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THE DEVELOPMENT OF MAGNOLIA AND LIRIODENDRON, INCLUDING A DISCUSSION OF THE PRIMITIVENESS OF THE MAGNOLIACEAE[1]

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(WITH PLATES I–III)

The recently announced theories of ARBER and PARKIN, especially as developed in their paper "On the origin of angiosperms" (2), make it desirable that more detailed work be done on the Magnoliaceae and related groups. Besides, as the embryo sac of only one species of the Magnoliaceae, *Drimys Winteri* (29), has ever been studied, it seemed probable that an investigation of other genera of this family, from this point of view, might be of value in several ways, but especially in furnishing either positive or negative evidence concerning the primitiveness of this family.

In the present study of *Magnolia virginiana* L. and *Liriodendron Tulipifera* L. two objects have been kept in mind: (1) the determination of the course of development of the sporogenous tissues and of the mature gametophytes, and also the examination of certain points concerning the gross structure and anatomy of these forms; (2) a consideration of the primitiveness of the Magnoliaceae on the basis of the evidence gained by such investigation, and of present prevailing theories respecting the origin of angiosperms.

The collection of material was begun in December of 1910 and was continued at intervals of generally two or three weeks until

[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, no. 34.

September 1911. Material for the study of *Liriodendron* was obtained at Homewood, the new site of the Johns Hopkins University, and that for *Magnolia* in a swampy region at Glen Burnie, Maryland, a typical habitat for the so-called swamp magnolia. Most satisfactory results were obtained by using a chromacetic solution for fixing, although acetic alcohol worked well also. The principal stains used for sporogenous tissues and young embryo sacs were iron-hematoxylin and orange G; and for older sacs safranin and Delafield's hematoxylin, or for embryos hematoxylin alone.

The writer is greatly indebted to Professor DUNCAN S. JOHNSON, and desires to express thanks for many suggestions and much helpful criticism during the progress of the work.

As the course of development is very similar in *Magnolia* and *Liriodendron*, in the following description attention will be directed mainly to the former, differences between the two species being pointed out where they occur.

The stamens of *Magnolia* and *Liriodendron* have elongated anthers on short filaments, the connectives extending beyond the anthers. The anthers contain four locules, with walls of 3 or 4 layers of cells, and dehisce longitudinally. In *Magnolia* the sporogenous tissue in the stamens is already differentiated early in December (fig. 15), and in *Liriodendron* early in January. Little change occurs in this tissue until March, when the sporogenous cells enlarge considerably and divide. The nuclei of the microspore mother cells are found in synapsis from April 20 to May 1 (fig. 16). The first mitosis in both species usually occurs during the first week in May (fig. 2), and tetrads are developed by the simultaneous method about May 10 (fig. 18).

After the first and second mitosis of the nuclei of the microspore mother cells the number of chromosomes is 19 (figs. 3 and 19). The actual number in the microspore mother cells, or in the vegetative tissues, was not determined, but is much greater than 19; therefore, as the usual heterotypic division occurs in the microspore mother cells, and after this division a smaller number of chromosomes than before is present, there can be little doubt that in microspore formation reduction takes place, and that the x and $2x$ generations are characterized respectively by 19 and 38 chro-

mosomes. Neither STRASBURGER (**29**) nor ANDREWS (**1**) determined the exact number of chromosomes in the forms studied by them.

The mature pollen grains are oval in shape, with rather thick walls. In the case of *Magnolia* they are binucleate (fig. 21), while those of *Liriodendron* are two-celled (fig. 4). This condition is found in the anthers before dehiscence occurs, which, in the latter species, is between May 15 and 18, and in the former about May 30.

In early stages of development it is evident that the tapetum arises from the sporogenous tissue (fig. 15), later differing from it especially in size of cells and nuclei (fig. 17). Still later, when tetrads are forming, the tapetum consists of 2 or 3 layers of large, binucleate cells lining the walls of the loculi (fig. 1). Finally, as the pollen grains mature, the tapetum gradually disappears, but there is no evidence of migration of its nuclei among the developing pollen grains (fig. 18). The walls of the loculi consist of 3 or 4 layers of cells. As the anthers mature, the subepidermal layer of cells becomes differentiated into the usual type of cells which are active in dehiscence.

The ovules in both *Magnolia* and *Liriodendron* are marginal in origin and anatropous, but there is considerable difference in the time of initiation of these organs. It occurs in the former as early as the first two weeks in December or earlier, while in the latter not until after the middle of March. Early in April, however, the condition attained is about the same in both, and the two develop at the same rate until the completion of the embryo sac. The first rudiment of the inner integument appears when the megaspore mother cell is well differentiated (April 20), and soon afterward the outer integument is initiated (fig. 5). From the archesporial cell (fig. 22) a tapetal cell is cut off (fig. 23), apparently in both species, and the mother cell soon becomes deeply buried within the nucellus (fig. 24). By two successive divisions of the megaspore mother cell 4 megaspores are formed (figs. 6, 7, 25, 26, 27), the innermost of these in each case being functional, while the others degenerate. By the time the 4 megaspores have developed the innermost one comes to lie very deep within the nucellus (fig. 7).

The development of the embryo sac is normal throughout.

Even in the binucleate condition the sac is considerably elongated (fig. 28); it continues to grow more in length than in breadth; finally it becomes slightly curved and also somewhat enlarged at the ends. The tetranucleate sac is shown in fig. 29. The mature sac is of the ordinary type, containing an egg and two beaked synergids at the micropylar end, three antipodals at the opposite end, and two endosperm nuclei somewhat above the middle (figs. 8, 9, 10, 30).

While fertilization was not observed in either species, there can be little doubt of its occurrence in both. The evidence for this is that when material of a certain age is examined the remains of the pollen tube are invariably found in the micropyle and also within the embryo sac (fig. 31).

In all the sacs of *Magnolia* in which the polar nuclei were found, partial fusion had already occurred (fig. 30). After fusion the endosperm nucleus moves close to the egg apparatus, and then, before division of the (presumably fertilized) egg, the first division of the fusion nucleus takes place. At this division a wall is formed transverse to the long axis of the embryo sac (fig. 31), hence the endosperm is cellular from the start, differing from *Drimys* (**29**) in this respect. Both of the resulting cells participate in the formation of the endosperm, that part of the sac surrounding the egg becoming filled with endosperm much more rapidly than the antipodal end. In early stages the endosperm is quite compact (fig. 32), but later the cells seem to enlarge; finally, in the mature condition, the nucellar tissue disappears completely and an abundant supply of compact endosperm fills the seed.

In the development of the embryo of *Magnolia* the first division is transverse, the second longitudinal to the long axis of the embryo sac (fig. 33). A second longitudinal wall separates the young embryo into octants (fig. 34), and soon thereafter the divisions apparently become quite irregular (fig. 35). There is much difference in the form of different embryos of approximately the same age, some being nearly globular (fig. 35) and others considerably elongated. A well defined suspensor (fig. 36) appears somewhat late and may persist until the embryo is mature. The cotyledons are initiated after the embryo has enlarged considerably.

They appear simultaneously and are independent from the start, there being no evidence of a pseudo-monocotyledonous habit such as occurs in various other members of the Ranales. In the mature seed the embryo is typically dicotyledonous, with a short radicle and well developed hypocotyl and cotyledons (fig. 37).

The seed coats of *Magnolia* have been described by different botanists, probably the earliest correct description being that given by ASA GRAY (10). In the mature seed the outer integument is differentiated into two layers, an outer fleshy one well filled with oil receptacles, and an inner stony layer of bony hardness. The inner integument forms only a thin layer in the ripe seed. After dehiscence of the carpels the seeds remain suspended a few days by means of an elastic thread formed from the spiral thickening bands of the xylem elements of the raphe. Finally the threads may be broken and the seeds fall to the ground; or sometimes the entire cones with most of the seeds still attached are shed. This shedding of the cones results from a break across the base of the peduncle, but without the formation of a definite absciss layer.

In the case of *Liriodendron* the development of the endosperm and embryo were not investigated.

Before discussing the embryo sac and certain other points mentioned above, several features of gross structure and anatomy deserve attention. ARBER and PARKIN (2) define the term flower as "a special form of a type of strobilus common to angiosperms and certain mesozoic plants," and propose to designate it as an anthostrobilus. The anthostrobilus differs from all other strobili in that it is typically amphisporangiate, with the megasporophylls above the microsporophylls on an elongated axis, and below the sporophylls a distinct perianth which is wholly or partially protective. The angiospermous type of anthostrobilus is called a euanthostrobilus, and is believed by them primitively to have possessed among others the following characteristics: a large or indefinite number of parts arranged spirally; ovules orthotropous, several in each ovary, with two integuments; marginal placentation; filaments short, bearing long anthers with the connectives prolonged beyond them; members of the perianth all similar, or more or less differentiated; entomophilous. The flowers of

Magnolia and *Liriodendron* differ from the above type only in having anatropous ovules and two-seeded carpels. In the proanthostrobilus or Bennettitean type of "flower" corresponding parts occur with a similar arrangement. ARBER and PARKIN hold that "its parts are homologous with the carpels, stamens, and perianth of a typical amphisporangiate angiospermous flower." It differs from this, however, "especially in the presence of a seminal pollen-collecting mechanism, and in the form of the microsporophylls," which are decidedly fernlike. More details regarding the proanthostrobilus cannot be included here, but may be found in the well known work of WIELAND (**32**).

The seedling structure of *Liriodendron* has been described by Miss THOMAS (**30**). She finds here a form intermediate between the normal tetrarch and diarch types of transition from cotyledons and hypocotyl to root as found in the Ranales, Rhoedales, and various other dicotyledons. The writer has confirmed Miss THOMAS' descriptions. The peculiarity in *Liriodendron* is that while in the cotyledons and upper part of the hypocotyl the structure is that ordinarily occurring in connection with the tetrarch type of root (figs. 13, 14), lower down in the hypocotyl certain elements disappear so that the root is diarch (fig. 12). On account of lack of material the seedling of *Magnolia* has not yet been studied.

In both gross structure and anatomy *Magnolia* and *Liriodendron* afford many points of similarity, possessing certain characters common to all Magnoliaceae and others which are peculiar to the Magnolieae (**28, 21**). Among the former are woody stems, alternate leaves, secretory cells, a characteristic type of stoma, and more or less abundant endosperm in the seed; the latter include foliar stipules, sclerenchymatous diaphragms in the pith, and the bundles of the petiole more or less fused in an irregular ring.

The arrangement of the vascular bundles in the peduncle of *Magnolia* and in the petioles of *Magnolia* and *Liriodendron* is shown in figs. 11, 38, and 40, and details of the petiolar bundle of *Magnolia* are represented in fig. 39. Considering the Magnoliaceae as a whole, the petiolar bundles are distributed either as in these two species or in the form of a crescent. PARMENTIER (**21**) regards

the different arrangements as correlated with the size of the leaves. WORSDELL (33) finds medullary bundles in the petioles of various species of *Magnolia*, and interprets these as reminiscences of a primitive, more scattered system in their ancestors. He believes also that the somewhat irregular system of bundles in the peduncle of *Liriodendron* and *Magnolia* indicates the same thing. If we accept the view that dicotyledons have been derived from monocotyledons, this explanation might seem more or less plausible; however, on the theory that monocotyledons are secondary, this interpretation could hardly be correct.

Present theories concerning the primitiveness of various types of angiospermous embryo sac will now be discussed. According to the archegonium theory of PORSCH (22), the 8-nucleate embryo sac is to be interpreted as the equivalent of two archegonia, a micropylar one represented by the egg apparatus, and a chalazal one represented by the three antipodals. Accepting this view, as has been pointed out by BROWN (3), "we might conceive of the embryo sac of *Peperomia* as really composed of four sacs, each of which gives rise to one archegonium." A similar explanation might hold also for certain other anomalous embryo sacs such as are found, for example, in the Pennaeaceae. PORSCH, however, in his paper considers only the 8-nucleate type.

This theory has been criticized from various standpoints. In the gymnosperms, for instance (3), in all species that form archegonia, the megaspore first produces a non-cellular stage of the gametophyte, and subsequently a cellular stage in all species that form archegonia; and it is in this cellular tissue that the archegonia are initiated and formed. So if we homologize the first two divisions of the ordinary angiospermous embryo sac with the free nuclear divisions of the gymnospermous prothallus, the shifting of the archegonia from the cellular to the non-cellular stage of the prothallus must be explained.

ERNST (9) admits that the archegonium theory would be *sehr bestechend* if within the 8-nucleate embryo sac the two groups of nuclei were always of nearly the same form. The numerous variations from the "normal" type, not only in the form of the egg apparatus and of the antipodal apparatus, but also in the

behavior of the polar nuclei, serve, he believes, better to refute than to support PORSCH's theory.

Other valid criticisms might be offered, but we need not do this here, since, even if we were to accept the theory, the question as to the most primitive type of angiospermous embryo sac would still remain unsolved.

In a recent publication (9) ERNST, after considering the course of development of the ordinary angiospermous embryo sac, as well as of such peculiar types as occur in *Gunnera macrophylla*, *Peperomia pellucida*, *P. hispidula*, and the Pennaeaceae, arrives at conclusions quite different from those published slightly earlier by COULTER (5). According to COULTER, the most important of the five nuclear divisions in the development of the ordinary embryo sac are the first two, which result in the formation of tetrads. COULTER asserts that, so far as we know, if fertilization is to occur later, the reduction divisions are not omitted; and that whether the number of divisions of the embryo sac mother cell is reduced from 5 to 4, or even to 3, the reduction divisions are never omitted. In such forms as *Peperomia*, in spite of the fact that the embryo sac contains 16 nuclei, there are only 4 divisions of the embryo sac mother cell, instead of 5 as in the ordinary 8-nucleate type of embryo sac.

To this view ERNST replies: "Die Entwicklungsvorgänge im Embryosack scheinen mir unabhängig von seiner Entstehung betrachtet werden zu müssen." So ERNST holds that although the omission of tetrad formation is a reduction of the course of development and not a primitive character, it has, nevertheless, no influence on the development of the embryo sac, and that the 5 divisions of the embryo sac mother cell resulting in the 8-nucleate sac belong to two entirely different phases of development; that is, formation of spores, characterized by reduction of chromosomes, and germination of spores, characterized by polarity, number of nuclear divisions, position of nuclei, development of vacuoles, and cell formation.

It is evident that COULTER regards the 16-nucleate embryo sac of such forms as *Peperomia* as derived by a reduction of the divisions of the embryo sac mother cell to 4 instead of 5. ERNST,

on the other hand, sees here an omission of tetrad formation and one more than the usual number of divisions in the germination of the megaspore to the embryo sac. He therefore considers the 16-nucleate sac as an older, or at any rate an independent, form of embryo sac, not derived from the 8-nucleate type. These two views, then, are directly opposed to each other.

With respect to the nature of the 16-nucleate embryo sac of *Peperomia*, CAMPBELL (**4**) and JOHNSON (**15, 16**), as is well known, have arrived at opposite conclusions. CAMPBELL says: "*Peperomia*, in regard to the embryo sac, probably represents the most primitive form yet discovered among the angiosperms. . . . . *Peperomia* offers a basis for an explanation of the homologies of the embryo sac." JOHNSON, a little later, after studying several genera of Piperaceae, maintained that the peculiarities in *Peperomia* are of secondary origin. The latter view is supported by BROWN (**3**) who, in *Peperomia sintenisii* and *P. arifolia*, finds that at the first two divisions of the embryo sac mother cell typical reduction of chromosomes occurs; and also that during these divisions the nuclei are separated by evanescent cell walls. So he concludes that these phenomena seem to indicate that the sac is a compound structure derived from the nuclei of four megaspores, the primary sac nucleus being a mother cell rather than a megaspore.

Reference has been made to COULTER'S view that while the genesis of the ordinary angiospermous embryo sac from the megaspore mother cell involves 5 divisions, the essential part of the process is found in the first two divisions which, so far as we know, are necessary if fertilization is to occur. BROWN (**3**) maintains that we cannot make chromosome reduction the sole criterion of megaspore formation, and adds as another distinction that while a division giving rise to megaspores is characterized by a cell wall or a cell plate, the first division of a megaspore is not accompanied by a cell plate. Quite lately SMITH (**27**) has pointed out that this distinction does not hold in the case of *Clintonia*, where, at the first division of the megaspore nucleus, a cell plate appears, and SMITH infers it will not hold for certain other cases.

Sufficient emphasis, it seems to the writer, has never been given to the fact that while the anomalous types of embryo sac are as a

rule of rare occurrence and are distributed among entirely unrelated families, on the other hand, the ordinary 8-nucleate type developed by 5 divisions of the megaspore mother cell occurs in nearly all families of angiosperms from the most primitive to the most specialized. This is significant and is hardly to be explained on any other hypothesis than that the latter type is primitive. We have in the 8-nucleate sac, it seems, a structure of marvelous constancy in development and arrangement of parts, evolved in the course of long ages, the exceptions to which only strengthen the view that it is a primitive type of embryo sac.

Another reason for regarding anomalous types of embryo sac as derived is the variability in the manner of their development. This is especially marked in the case of 16-nucleate sacs, where a peculiar method of development is found in almost every new case discovered. Of course it may be objected that these variations are of minor importance. But even if this were true, it certainly contrasts strikingly with the remarkable uniformity so very general even in minor details of the development of the ordinary angiospermous type, and is a fact to be explained.

Whether or not we consider the first 4 nuclei produced by division of the embryo sac mother cell as megaspores, and this view seems reasonable in most cases, is probably of less importance than many have believed. The genesis of the angiospermous sporophyte, aside from the exceptional cases such as those involving apogamy and budding, always begins with a perfectly definite structure, the embryo sac mother cell. If fertilization is to occur later, then, without exception, reduction of chromosomes takes place at the first two divisions of this cell. Now, considering parthenogenesis as a secondary phenomenon, we see that in the development of all normal embryo sacs (that is, those capable of being fertilized), whatever their type of mature structure, there is this common feature in development. After the reduction divisions the embryo sac may develop as the product of one-fourth, one-half, or all of the four reduced nuclei derived from the original mother cell. The ordinary 8-nucleate type develops as the product of one-fourth of these nuclei by three successive divisions; the sac of *Cypripedium* (**20**) develops as the product of one-half of these

nuclei by one division; that of *Lilium* as the product of all of these nuclei by one division; and that of *Peperomia* from all of these nuclei by two divisions. The three latter cases clearly show an abbreviation in the course of development of the gametophyte, whether we regard that course as beginning with the embryo sac mother cell or after chromosome reduction.

That we cannot make chromosome behavior the sole criterion for distinguishing sporophyte and gametophyte is doubtless true. This is evident from chromosome behavior in cases of parthenogenesis such as occur in *Alchemilla* (19), and in the numerous instances of apogamy and apospory among both pteridophytes and spermatophytes. However, it is probable that no one has ever dreamed of making such unusual phenomena the basis of any theory of the nature or phylogeny of the angiospermous embryo sac. These phenomena undoubtedly are secondary. Hence the conception that chromosome behavior is the most important criterion, at least in all cases where fertilization occurs, seems well founded. So if abbreviation in the developmental history of the gametophyte in angiosperms expresses an evolutionary tendency which can be traced back as far as the gametophyte of the pteridophytes, then anomalous embryo sacs are secondary rather than primitive types.

If now the embryo sacs of those Magnoliaceae thus far investigated are considered, no clue is discovered in their development or structure as to the primitiveness of the group; and this for the simple reason that, although we regard this type as the most primitive among angiosperms, yet the same type is the common one among all angiosperms. Moreover, whatever theory we accept, the past study of the embryo sac has served mainly to emphasize the vast difference between angiosperms and lower groups of plants in this respect, and so to increase rather than to bridge the wide gap between them. It would seem then that if the problem of the origin of angiosperms is to be solved this must come about principally as a result of investigations of other features than the embryo sac.

During the last two decades the amount of purely descriptive literature dealing with embryo sacs has grown to huge proportions,

but the different theories as to which type is primitive have not been reconciled. If, however, anomalous types of angiospermous embryo sacs are secondary and not primitive, as seems most reasonable, then the causes of these secondary modes of development should be investigated. This is obviously a problem presenting serious difficulties, yet they are probably not insurmountable. A key to the situation may probably be found by considering the possibility that there is some relation between anomalous types of embryo sac development and peculiar environmental conditions. This has been suggested, for example, by JOHNSON (15).

Since the strobilus theory of ARBER and PARKIN (2) is based so largely on a comparison of the flower (euanthostrobilus) of angiosperms and the proanthostrobilus of the Bennettitales, we must refer to certain views as to the nature of these structures. ARBER and PARKIN, agreeing with HALLIER (11) and SENN (26), consider the amphisporangiate flower of *Magnolia* and *Liriodendron* as made up of sporophylls and perianth borne directly on the main axis of the floral shoot rather than as a compound structure. This they regard as the most primitive type of angiospermous flower, reproducing the essential features of the Bennettitean strobilus. WETTSTEIN (31) and others, on the contrary, think that the primitive type is to be sought for among the monosporangiate Apetalae, and that the angiospermous fructification is a reduced inflorescence, derived from that of the gymnosperms. LIGNIER (17), at least, interprets the Bennettitean strobilus also as a compound structure, an inflorescence. It is clear that conclusions as to the primitiveness of different groups, as well as to the origin of angiosperms as a whole, will vary according to which of these views is accepted.

The greatest differences between the two theories have been indicated. Whether the primitive angiospermous flower was anemophilous or entomophilous is perhaps of relatively minor importance, yet this question also is involved in both of these theories. It would seem natural to imagine anemophily as the method of pollination among primitive angiosperms, yet if entomophily has played as important a rôle as many suppose in their evolution, from their very origin, then the latter must be primitive for this group.

Thus far the monocotyledons have been left out of account in looking for primitive angiosperms. Let us now turn to this group.

The remarkable uniformity in the development and mature condition of the male and female gametophytes of monocotyledons and dicotyledons has been brought forth repeatedly as the strongest argument in favor of a monophyletic theory. In spite of objections such as the claim that "similarity in structure may be the outgrowth of the changes that resulted in the evolution of seeds" (6), it seems that we are far from the point of even thinking of abandoning this as well-nigh irrefutable evidence of close genetic relationship between the two great classes of angiosperms.

Within the last few years the striking similarity in the seedling structure of dicotyledons and monocotyledons has been demonstrated in many forms. Although the generalization that "ontogeny repeats phylogeny" has likely been overworked, yet if there is anything at all in this rule, then the evidence from seedling structure deserves its full share of consideration.

In favor of the view that the two groups have originated independently are the differences in their anatomy, and in the development and mature condition of the embryo. As a result of recent study, however, we learn that anatomically the structure of seedlings in particular, but also of mature individuals in the two groups, offers many striking points of resemblance. Other differences, generally regarded as secondary in importance, are seen in the venation of the leaves, in the grandifoliate as opposed to the parvifoliate habit, and in floral symmetry.

The principal resemblances and differences between monocotyledons and dicotyledons on which present phyletic theories are based have been mentioned. In reviewing these theories we may recall the fact that while according to most if not all monophyletic theories proposed until recently, dicotyledons were assumed to be derived from monocotyledons, students of phylogeny today quite generally hold the opposite view. WORSDELL (33), however, is still inclined to the view that monocotyledons rather than dicotyledons are primitive. He believes that "angiosperms have developed directly from an ancestor belonging to the bryophytic level, and that they

have not come from either gymnosperms, pteridosperms, or ferns." His conclusion is based mainly on the following insufficiently substantiated assumptions: that taking the vegetable kingdom as a whole, the grandifoliate habit is primitive, the parvifoliate derived; that the appearance of two cotyledons in dicotyledons is illusive, there really being only one which is deeply bifurcated; that in the monocotyledonous seedling there is no room for the scattered arrangement of the bundles, so we cannot on account of its absence here conclude that the scattered condition has been derived from a vascular cylinder. These points cannot be discussed here; it suffices to say that the criticism that this view assumes entirely too much seems fair. Besides, the evidence from fossils is entirely against it, the general conclusion of paleobotanists being that from the Bryophyta no higher forms have ever evolved. SCOTT (**25**), touching this point, says: "neither among living nor fossil plants has any indication of a structure intermediate between the plant of a vascular cryptogam and the fruit (sporophyte) of a bryophyte ever been discovered."

The view that monocotyledons have been derived from dicotyledons, doubtless at a rather early period in the history of angiosperms, and possibly by branching from several points along the dicotyledonous line, has been much strengthened by the researches of the last 15 years. Miss SARGANT (**24**) regards the common characters of monocotyledons and dicotyledons as too numerous and uniform to have been acquired independently, and emphasizes the fact that angiosperms are especially unique with respect to their flowers, carpels, and endosperm. The attainment of practical identity in the "germination of the embryo sac and the history of the endosperm" by independent evolution among monocotyledons and dicotyledons "would require a series of coincidences," she says, "so improbable as to be inconceivable." With respect to the flower, Miss SARGANT agrees with the view of ARBER and PARKIN given elsewhere.

Probably Miss SARGANT'S most important contribution to the monophyletic theory is based on anatomical investigations. The presence or absence of a cambium seems to account largely for the difference in detail between monocotyledonous and dicotyledonous

stems, hence the great importance of any evidence as to its presence among primitive angiosperms. That a true cambium develops in certain monocotyledons (*Gloriosa*, **23**) is now well known; but especially significant is the fact that while the structure of the mature stem of monocotyledons and dicotyledons generally varies greatly, the primary structure of the dicotyledonous stem, which is the same in seedlings and mature plants, is also frequent in monocotyledonous seedlings. So in the light of this evidence and also of the fact that a cambium is usually present among living gymnosperms and extinct vascular cryptogams, and is universally present, so far as known, among extinct gymnosperms, the view that primitive angiosperms possessed a cambium seems well founded.

The argument for most of Miss SARGANT'S other views cannot be included here, yet one other conclusion should be mentioned. Since among gymnosperms monocotyledonous forms are unknown, and the dicotyledonous condition prevails, one would naturally presume that the embryo of primitive angiosperms was dicotyledonous; besides, the embryological development of angiosperms points in the same direction. So the general conclusion is that monocotyledony is secondary, being the result of a fusion of two cotyledons.

ARBER and PARKIN in discussing the origin of angiosperms hold "that monocotyledons branched off from the main angiospermous line, that is, dicotyledons, at a very early period." Since the embryo of *Bennettites* was dicotyledonous, they regard the Hemiangiospermae as dicotyledonous also, and so conclude that the monocotyledonous type is the less primitive one. In their opinion Miss SARGANT'S explanation of the monocotyledonous embryo is the best yet offered. They also recognize the need of accounting for the origin of the monocotyledonous habit.

For a number of years the so-called anomalous dicotyledons have attracted students in the hope that knowledge of their embryos and anatomy would aid in solving phylogenetic problems. One of the general conclusions of such investigations is that the anomalous characters are secondary and not primitive features. MOTTIER (**18**), for example, says, "it is probably true without

exception that dicotyledonous plants possessing anomalous embryos are either partially or wholly geophilous in habit, having stems either in the form of a rhizome, tuber, or a short, squat axis."

That anomalies not only in embryology and anatomy, but even in embryo sacs, depend largely on environmental conditions has been suggested in the past (JOHNSON 15), and seems continually to be receiving wider attention. HILL (14), for example, has discovered in the Andes, Central America, and Mexico a few geophilous species of *Peperomia* which are of great interest. Although their seedlings are described as possessing all the external characters of monocotyledons, yet they are true dicotyledons, but the cotyledons exhibit a marked division of labor, one serving for absorption, the other for photosynthesis. That these species are true dicotyledons is shown by the structure of the seed; the presence of stomata on the lamina of the absorbent cotyledon; persistence of the primary root for some time after the formation of the bulb; and the vascular structure of the seedling. Moreover, most of the members of the genus, containing some 400 species, are normally dicotyledonous. The peculiar habit of these few species is therefore interpreted as due to xerophytic conditions, resulting in the assumption of the geophilous habit, accompanied by formation of bulbs or tubers, and finally affecting even the embryonic structure of the plants so that the division of labor referred to above has resulted. Although HILL opposes Miss SARGANT'S theory as to the origin of the single cotyledon of monocotyledons, it is worthy of note that he attributes the anomalies in *Peperomia* to the geophilous habit. This is the same cause that other workers have assigned for the pseudo-monocotyledonous habit, anomalous stem structure, and so on, in the case of various other dicotyledons.

Now if it is conceivable that a pseudo-monocotyledonous habit has arisen in different ways among dicotyledons, then the possibility of the same thing happening among monocotyledons presents itself, especially if monocotyledons have branched off at several points along the dicotyledonous line. Indeed, attempts at a causal explanation of the origin of monocotyledons from dicotyledons have actually been made, one of the most important of these being HENSLOW'S theory (12).

This theory is founded mainly on the large number of coincidences among both dicotyledonous and monocotyledonous aquatic plants, and on the fact that all terrestrial monocotyledons exhibit the same coincidences. HENSLOW, as well as many others, regards the monocotyledons as degenerate (when compared with dicotyledons), although there is not always agreement as to the cause of degeneracy. HENSLOW himself believes an aquatic habit has been the principal cause, and points to a number of characteristics of monocotyledons and dicotyledons that he considers the result of adaptation to a moist or aquatic environment. Among these peculiarities are large size of leaves, water storage organs, the pseudo-monocotyledonous condition of certain dicotyledons, early loss of primary root, and "endogenous" arrangement of cauline bundles. HENSLOW remarks: "In the title of my first paper in 1892 (13), I used the word 'theory,' but . . . . I feel justified in abandoning the term; for I would maintain that the conclusion has passed the stage of hypothesis and probability only, to that of a *demonstrated fact*." While it is likely that very few of us would be willing to subscribe to this conclusion, yet all must agree that such considerations are extremely suggestive, and indicate a line of work that is promising, especially if taken up experimentally.

To multiply examples would probably not add to the force of the argument. We see that various competent workers attribute anomalies among dicotyledons to a geophilous habit, response either to xerophytic or to hydrophytic conditions. Such responses result in structural peculiarities in stems, formation of various types of underground stems, a pseudo-monocotyledonous habit, or division of labor among the cotyledons. When we turn to the monocotyledons, we find these peculiarities duplicated, but as a rule they are intensified. That their production is related to environment seems clearer in the case of dicotyledons than of monocotyledons, no doubt because many monocotyledons at present live where the prevailing conditions do not seem to necessitate geophily. The persistence of these peculiarities in such environments may be interpreted as retention of past characters.

It seems then that, on account of many similarities between

monocotyledons and anomalous dicotyledons, HENSLOW'S conclusion that the former have been derived from the latter as a response to the same factors that determined the geophilous habit is reasonable, at least, as a working hypothesis. Failure to recognize the fact, however, that geophily may express itself variously has sometimes led to disagreement in theories where none actually exists. This appears especially in discussions of the origin of the monocotyledonous from the dicotyledonous habit, it being held that monocotyledony has arisen in only one way. There are at least three plausible theories concerning the method by which this may have occurred: first, by suppression of one of two cotyledons; next, by fusion of two cotyledons; and, finally, by a division of labor between two cotyledons. Now since we find a difference in the behavior of the cotyledons in certain anomalous dicotyledons, it is entirely probable that the same thing has happened in the origin of monocotyledons from dicotyledons, so much the more so if there is more than one monocotyledonous branch from the primitive dicotyledonous stock.

While, as has been shown, the most generally accepted view is that angiosperms are monophyletic we must also remember the possibility of a diphyletic origin. COULTER (6) has expressed his view on this point as follows: "In our judgment the evidence is strongly in favor of the independent origin of the two groups, which have attained practically the same advancement in the essential morphological structures, but are very diverse in their more superficial features. Their great distinctness now indicates either that they were always distinct or that they originated from forms that were really proangiosperms and neither monocotyledons nor dicotyledons." Those who hold this view will have to explain more satisfactorily than has been done the similarity between monocotyledons and dicotyledons in gametophytic development and in seedling structure; the general similarity between monocotyledons and anomalous dicotyledons; and the evidence that primitive angiosperms possessed a cambium and were dicotyledonous. The monophyletic theory has been strongly reinforced in recent years and the writer finds it more acceptable than the diphyletic, but more evidence is needed before it can be unreservedly accepted.

It is evident then, from the above considerations, that we regard the Magnoliaceae, since they belong to the more primitive group of angiosperms, as more primitive than the monocotyledons. Let us next consider certain theories relative to the primitiveness of various features among the dicotyledons themselves.

Present theories on this subject are fairly well represented by the views of WETTSTEIN (31) on the one hand and those of ARBER and PARKIN (2) on the other. Both believe that monocotyledons have been derived from dicotyledons. WETTSTEIN holds, because of similarity in cotyledons, stem, floral structure, and reduction of the primary root, that monocotyledons have been derived from the Polycarpicae. He also points out that while we may think of derivation of one cotyledon from two, the reduction of a primary root, and so on, the opposite would not seem possible under any circumstances. So he concludes that we must turn to the dicotyledons in considering the phylogeny of angiosperms, but disagrees entirely with many as to which dicotyledons are most primitive.

Both theories derive angiosperms from gymnosperms. According to WETTSTEIN, primitive angiosperms should present among others the following characteristics: prevalence of woody plants and absence of vessels in the vascular bundles; prevalence of monosporangiate flowers, with either no perianth or one of simple structure; prevalence of anemophily. These are gymnospermous characters, and WETTSTEIN holds that we should regard that group of angiosperms as most primitive which exhibits these characters developed in high degree. ARBER and PARKIN (2), HALLIER (11), and others give a much longer list of characters which they believe are primitive. According to their views, amphisporangiate, actinomorphic flowers, with elongated axes bearing numerous free, spirally arranged floral parts, are primitive. Such flowers also possess a well developed, undifferentiated perianth and are entomophilous. Besides, primitive angiosperms are dicotyledonous, have small embryos and abundant endosperm, are treelike, and lack true vessels among autophytic species.

If it is granted that all the essential characters that may be regarded as primitive have been included in these lists, then it is evident that the great differences between the two theories relate

to but a few points, points which involve, however, no end of difficulty. The two theories would apparently be reduced to one if we could say which of the following are primitive: monosporangiate or amphisporangiate flowers; presence or absence of a perianth; anemophily or entomophily. Of possibly less importance is the question whether the angiospermous flower is a modified simple (that is, unbranched) shoot or a modified infloresence. There is agreement as to the primitiveness of such characters as actinomorphy, freedom of floral parts, dicotyledony, lack of true vessels in the vascular strands, and prevalence of woody plants. So it seems important to discuss the differences cited.

The fact that certain characters are common to all or nearly all gymnosperms seems in many instances to be one of the strongest reasons for regarding those characters as primitive if they occur at all among angiosperms. Among these supposedly primitive characters are dicotyledony, prevalence of woody plants, and absence of tracheae in the conducting strands. For the same reason we might conclude that primitive angiosperms were anemophilous and possessed naked, monosporangiate flowers, since these characters also are common to most gymnosperms.

Before discussing this matter further, reference may be made to the possibility of there being two entirely separate lines of dicotyledons. Reasons have been given for believing that angiosperms are monophyletic. Now if the similarity between dicotyledons and monocotyledons is close enough to warrant such a conclusion, then, since the similarity is so much more striking among dicotyledons themselves, this conclusion seems all the more certain in the latter case. In the group Dicotyledoneae the diversity in the development and structure of the gametophytes and embryos is surely less marked than is the diversity among angiosperms as a whole, and so difficulties are increased accordingly if any other than a monophyletic theory is proposed for the phylogeny of dicotyledons. It is certainly true that the reproductive structures and organs of spermatophytes are among their least plastic features. While it is dangerous to emphasize too strongly the importance of even the most stable character to the exclusion of others, nevertheless, if the view that the embryo sac points unmistakably to a

monophyletic origin of angiosperms is incorrect, a more satisfactory one has never been advanced. The homoplastic explanation seems well-nigh inconceivable. Besides, in the morphological and anatomical structure of seedlings and mature plants all dicotyledons are essentially alike, so most of the important differences, as has been indicated above, are in the flowers. Taking all the evidence into account then, it seems likely that all dicotyledons are of one stock, and that the monocotyledons have arisen as one or more branches of this stock.

If then dicotyledons are monophyletic, which are the most primitive? If, as ARBER and PARKIN (**2**), WETTSTEIN (**31**), and others suppose, they have been derived from gymnosperms, from what particular gymnospermous stock may they have come? The Gnetales and more recently the extinct Bennettitales have each been thus designated. Even if dicotyledons originated from a gymnospermous stock, opinions will differ as to which is the parent group, depending on whether one regards naked, monosporangiate, anemophilous flowers, or entomophilous, amphisporangiate ones with a perianth as primitive.

The entomophilous habit seems clearly associated with much in the evolution of angiosperms, but it does not necessarily follow that primitive angiosperms were entomophilous. That anemophilous angiosperms may succeed and persist in competition with entomophilous forms is well illustrated by such groups as the Amentiferae and Gramineae.

Few, if any, believe that the flower of any existing angiosperm is like the primitive angiospermous flower. Whether this primitive flower was monosporangiate or amphisporangiate it does not follow, though this is quite possible, that any particular flower of today is the direct descendant of a similar type in its ancestor. It is certain that in many instances amphisporangiate flowers have become monosporangiate, and that perianths have been more or less completely lost.

That the opposite may have occurred, however, is less easily proven. ARBER and PARKIN (**2**), for example, object to ENGLER's view (**8**) that the monosporangiate Apetalae are primitive among dicotyledons, by saying that "it must be assumed that the perianth

is evolved *de novo* and is an organ *sui generis*." But suppose we do assume that primitive angiosperms possessed a perianth, the origin of the perianth still remains to be explained. Even if we say that it is a direct derivative from a Bennettitean ancestor, its phylogenetic origin still remains a mystery. So if a perianth developed in some manner or other among the Bennettitales, might not the same thing have occurred among angiosperms, even long after they became a distinct group of plants?

The question whether primitive angiospermous flowers were monosporangiate or amphisporangiate presents even greater difficulties than that concerning the primitiveness of the perianth. If angiosperms have descended from gymnosperms we would rather expect primitive flowers to be monosporangiate. Evidence from gymnosperms that amphisporangiate flowers are primitive rests almost entirely on a single, extinct, much specialized group of plants, the Bennettitales. This group no doubt represents, as COULTER (7) suggests, "the end of a gymnosperm phylum." Moreover, that the proanthostrobilus of the Bennettitales corresponds closely to such a flower as that of *Magnolia* or *Liriodendron* still remains undecided. The resemblance is remarkable, yet if the view (17) that the Bennettitean inflorescence is a compound structure is correct, and if the flower of *Magnolia* is not compound, then the resemblance becomes only a superficial one.

If then we go back far enough in the evolution of angiosperms, the probability seems strong that the group was monosporangiate. Amphisporangiate flowers are unknown below angiosperms except in the Bennettitales and possibly *Welwitschia*. Although in a number of respects angiosperms and Gnetales have developed along parallel lines, it is now generally believed that the Gnetales are not transition forms leading to angiosperms. This view, however, does not preclude the possibility of common ancestry in the distant past. The Gnetales likely represent the end of a gymnospermous phylum just as the Bennettitales do. So neither group represents the direct progenitors of angiosperms.

COULTER and CHAMBERLAIN (7) say: "It is recognized that in the evolution of strobili among gymnosperms there were probably two distinct tendencies: a monosporangiate strobilus (Cyca-

dales, Cordaitales, Ginkgoales, Coniferales), and a bisporangiate strobilus with the anthostrobilus arrangement of sporophylls (Bennettitales, Gnetales, and leading to angiosperms)." This view apparently involves the idea that the Bennettitales, Gnetales, and angiosperms belong to one stock, but the relationship between the three groups at their origin may have been anything but close. Even though angiosperms are prevailingly amphisporangiate today this may not have been the condition among their remote ancestors; in fact it seems probable that those ancestors possessed monosporangiate flowers.

Though primitive angiosperms possessed monosporangiate, naked, anemophilous flowers, it is evident that they did not become the dominant group of plants until they developed amphisporangiate flowers with a perianth, and became entomophilous. This view is evidently opposed to the one that the present day angiosperms have been derived from the Bennettitean stock. The main reason for this belief is that except for resemblance in the fructifications, which may be quite superficial, the two groups are entirely different in structure. Indeed, had the Bennettitean proanthostrobilus never been discovered, probably no one would have ever suspected close relationship between such widely different groups. Leaving out of consideration the nature of the inflorescence, the following may be noted with reference to the Bennettitales: their seeds are of the gymnospermous type; the microsporophylls, microsporangia, and ramentum are fernlike in character; the external appearance and anatomy of the stem and leaf indicate relationship with cycads. All these characters suggest relationship with Cycadofilicales, while their strobili alone indicate a possible connection with angiosperms. It seems on the whole much simpler and safer to conclude that the Bennettitean proanthostrobilus and the angiospermous anthostrobilus are nothing more nor less than the results of homoplastic development, and that if they indicate relationship at all, it must be of the remotest kind, dating from a time prior to the origin of the Bennettitales as a separate stock, a time when neither true Bennettitales nor angiosperms had ever existed.

If then angiosperms were primitively anemophilous with naked

monosporangiate flowers, why are the present Monochlamydeae not to be regarded as the most primitive living members? This is because various features of the Monochlamydeae indicate reduction and not primitiveness. If, for example, the entire group as constituted by WETTSTEIN (31) be considered, it is found to be prevailingly syncarpous. This surely is not a primitive feature. Again, in various families there are closely related genera, even species, some of which possess a perianth, while in others it is rudimentary or entirely absent. It is difficult, in such cases at least, to conceive of the latter condition as primitive. Besides, the stamens and carpels may vary in number even in closely related genera and species. That the smaller numbers in such cases are derived seems to be a reasonable conclusion. Moreover, the inflorescences among the Amentiferae, for example, are compound structures exhibiting considerable complexity. This too can be more readily interpreted as derived and not primitive. Amphisporangiate flowers also occur in certain members of at least 7 families included by WETTSTEIN among the Monochlamydeae. Such cases add much weight to the view that the monosporangiate condition throughout the group is secondary and not primitive.

We may conclude then that considering existing angiosperms, the evidence at present available is in the main opposed to the view that they have been derived from forms at all closely related to the Bennettitales. The one striking similarity between modern angiosperms and the Bennettitales may well be a result of homoplasy. Among existing angiosperms, assuming that they are monophyletic, the derivation of forms having monosporangiate, naked flowers from those possessing amphisporangiate flowers, bearing an undifferentiated perianth, seems far simpler than the reverse. So, while agreeing with ARBER and PARKIN, HALLIER, and SENN in general with reference to those features which they believe are primitive among existing angiosperms, there seem to be no very adequate grounds for concluding that primitive angiosperms were provided with entomophilous, amphisporangiate flowers bearing a perianth. The opposite seems much more probable. If the latter is true, scarcely a suggestion of relation-

ship with the Bennettitales remains. But even if the former is true, it would seem hazardous to hold that angiosperms are more closely related to the Bennettitean stock than to any other gymnospermous stock. COULTER and CHAMBERLAIN (7) say that "the Cycadofilicales are so fernlike in every feature except their seeds, that their derivation from some ancient fern stock (called provisionally Primofilices) is as certain as phylogenetic connections can be. The origin of the Cordaitales therefore presents two alternatives: either they arose independently from the same ancient fern stock, or they were differentiated from the Cycadofilicales very early." The same two alternatives present themselves, it seems, in the case of angiosperms. Which view we accept is of little consequence since probably neither can be proven. Either view would make the connection between angiosperms and the Bennettitales, as we know them, a most distant one.

Let us now turn to our original question concerning the primitiveness of the Magnoliaceae among existing angiosperms. While the following list of characters of the Magnoliaceae is incomplete, it doubtless includes most of the more important ones that may be considered primitive: (1) the ordinary 8-nucleate type of embryo sac; (2) dicotyledony; (3) undifferentiated perianth; (4) amphisporangiate flower; (5) entomophily; (6) elongated conical floral axis; (7) actinomorphy; (8) indefinite number of free floral organs arranged spirally; (9) hypogyny; (10) apocarpy; (11) woody stems; (12) occasional absence of tracheae in the vascular bundles.

It is quite generally agreed that the last 7 of these characters are relatively primitive wherever found among angiosperms, or if not that they are of minor importance as evidence of phylogeny. The first two characters, since they are common to nearly all dicotyledons, are valueless as criteria for determining primitiveness. There are left three characters which may be either primitive or derived, namely, undifferentiated perianth, amphisporangiate flowers, entomophily. These three characters have no doubt developed together and are closely bound up with the evolution of angiosperms. If not, then naked, monosporangiate, anemophilous flowers must indicate primitiveness where found in existing

forms. We believe, for reasons previously given, that the type of flower found among the Magnoliaceae is primitive.

The present investigation adds little toward the solution of the plexus of problems involved in the origin of angiosperms and the relative primitiveness of existing groups. An opportunity was offered, however, to determine whether such a study as the present one of a possibly primitive group of angiosperms might yield any results of either positive or negative value as a contribution to present theories; to suggest new points of view and especially to emphasize certain old ones; to review briefly some of the principal theories of the present day on the primitiveness and origin of monocotyledons and dicotyledons; and finally to criticize where it seemed this might be helpful in bringing about in the future reinforcement or correction either of earlier theories or of the views expressed in this paper.

Summary

1. In both *Magnolia* and *Liriodendron* the sporogenous tissue in the anther is differentiated early in the winter. Tetrads develop by the simultaneous method and the pollen grains when mature are binucleate in *Magnolia*, two-celled in *Liriodendron*. The tapetum originates from the sporogenous tissue.

2. The x number of chromosomes in each species is 19.

3. The ovules in both species are marginal, anatropous, and provided with two integuments. The megaspore mother cell in each species, by two successive divisions, produces 4 megaspores, of which the innermost is functional. The mature embryo sacs are of the ordinary 8-nucleate type and fertilization probably occurs as usual.

4. The endosperm of *Magnolia* is cellular from the beginning of its formation and is abundant in the mature seed, surrounding a small, typically dicotyledonous embryo. The first division in the development of the embryo is transverse, the second longitudinal to the long axis of the embryo sac. The embryo has a well defined suspensor and no evidence of monocotyledony was found.

5. The seed of *Magnolia* possesses three coats: an outer fleshy

and within this a stony one, both developed from the outer integument, and a thin inner one from the inner integument.

6. The flowers of both species differ from the euanthostrobilus of ARBER and PARKIN only in having anatropous ovules and two-seeded carpels.

7. In *Liriodendron* the seedling possesses in the cotyledons and upper part of the hypocotyl the structure generally found associated with tetrarch roots, but the root is diarch.

8. All Magnoliaceae possess certain common anatomical characters, while others are peculiar to particular groups. The bundles in the petioles and peduncles of *Liriodendron* and various species of *Magnolia* are somewhat scattered, a feature the interpretations of which vary.

9. PORSCH's archegonium theory gives no suggestion as to what type of angiospermous embryo sac is primitive.

10. A consideration of all available evidence in the light of present theories very strongly favors the conclusion that the ordinary 8-nucleate type of angiospermous embryo sac is the most primitive. But in view of its wide distribution among all groups of angiosperms, its occurrence either among the Magnoliaceae or in any other family is no evidence of the primitiveness of that family.

11. Angiosperms are believed to be monophyletic, especially on account of the uniformity in the development and mature condition of the gametophytes, and because of similarity in seedling structure of monocotyledons and dicotyledons.

12. The view that dicotyledons have been derived from monocotyledons, as advanced by WORSDELL, rests too largely on assumption.

13. The theory that monocotyledons originated from dicotyledons is supported by evidence from gametophytic development and anatomical structure of seedlings and mature plants, as well as by indications that primitive angiosperms possessed a cambium and were dicotyledonous, and by the conclusion that the peculiarities of anomalous dicotyledons are secondary. The important differences between monocotyledons and anomalous dicotyledons on the one hand, and ordinary dicotyledons on the other, are believed

by many to be due to response to the peculiar environmental conditions surrounding the former.

14. Theories differ especially as to whether amphisporangiate, entomophilous flowers bearing a perianth, or naked, anemophilous, monosporangiate ones are primitive among dicotyledons. The writer holds, for reasons given, that in the remote past the ancestors of the dicotyledons (and so of all angiosperms) possessed naked, unisexual flowers, but that among existing groups hermaphrodite flowers provided with a perianth are primitive, and that the naked, unisexual forms existing today have been secondarily derived from the latter; moreover, that the appearance of entomophily, the amphisporangiate condition, and the perianth have been very important features in the evolution of modern angiosperms.

15. The Bennettitales, Gnetales, and angiosperms may have had common ancestors if we go back to a time prior to that when the Bennettitales became a distinct line. It seems reasonable to conclude either that angiosperms were derived from the same ancient fern stock from which the Cycadofilicales originated, or else that they were differentiated from the Cycadofilicales at a very early time.

16. In conclusion, it is believed that the most primitive of existing angiosperms are to be found among the Magnoliaceae or related forms, and not among forms with naked, monosporangiate, anemophilous flowers.

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EXPLANATION OF PLATES I–III

All drawings were made with the aid of a camera lucida from microtome sections.

Abbreviations used: *a*, antipodal; *ar*, archesporium; *cb*, cortical bundle; *cc*, central cylinder; *c*, cotyledon; *e*, egg; *em*, embryo; *es*, endosperm; *fvb*, fibrovascular bundle; *ii*, inner integument; *m*, mechanical tissue; *mgmc*, megaspore mother cell; *mg*, megaspore; *mcmc*, microspore mother cell; *mp*, micropyle; *n*, nucellus; *oi*, outer integument; *p*, pith; *pc*, parietal cells; *ph*, phloem; *pn*, polar nucleus; *pt*, pollen tube; *st*, sporogenous tissue; *su*, suspensor; *sy*, synergid; *t*, tapetum; *tc*, tapetal cell; *te*, tetrad; *v*, vacuole; *x*, xylem.

Liriodendron

FIG. 1.—Part of longitudinal section of microsporangium and wall; wall somewhat diagrammatic; ×350.

FIG. 2.—Pollen mother cells dividing; ×350.

FIG. 3.—Section of pollen grain with reduced number of chromosomes; ×600.

FIG. 4.—Section of two-celled pollen grain; ×350.

FIG. 5.—Longitudinal section of young ovule; ×300.

FIG. 6.—Megaspore mother cell divided; ×350.

FIG. 7.—Longitudinal section of ovary showing tetrad of megaspores deeply buried within nucellus; ×350.

FIG. 8.—Longitudinal section of micropylar end of embryo sac; ×350.

FIG. 9.—Polar nuclei fusing; ×350.

FIG. 10.—Longitudinal section of antipodal end of embryo sac; ×350.

FIG. 11.—Transverse section of petiole showing arrangement of fibrovascular bundles; ×50.

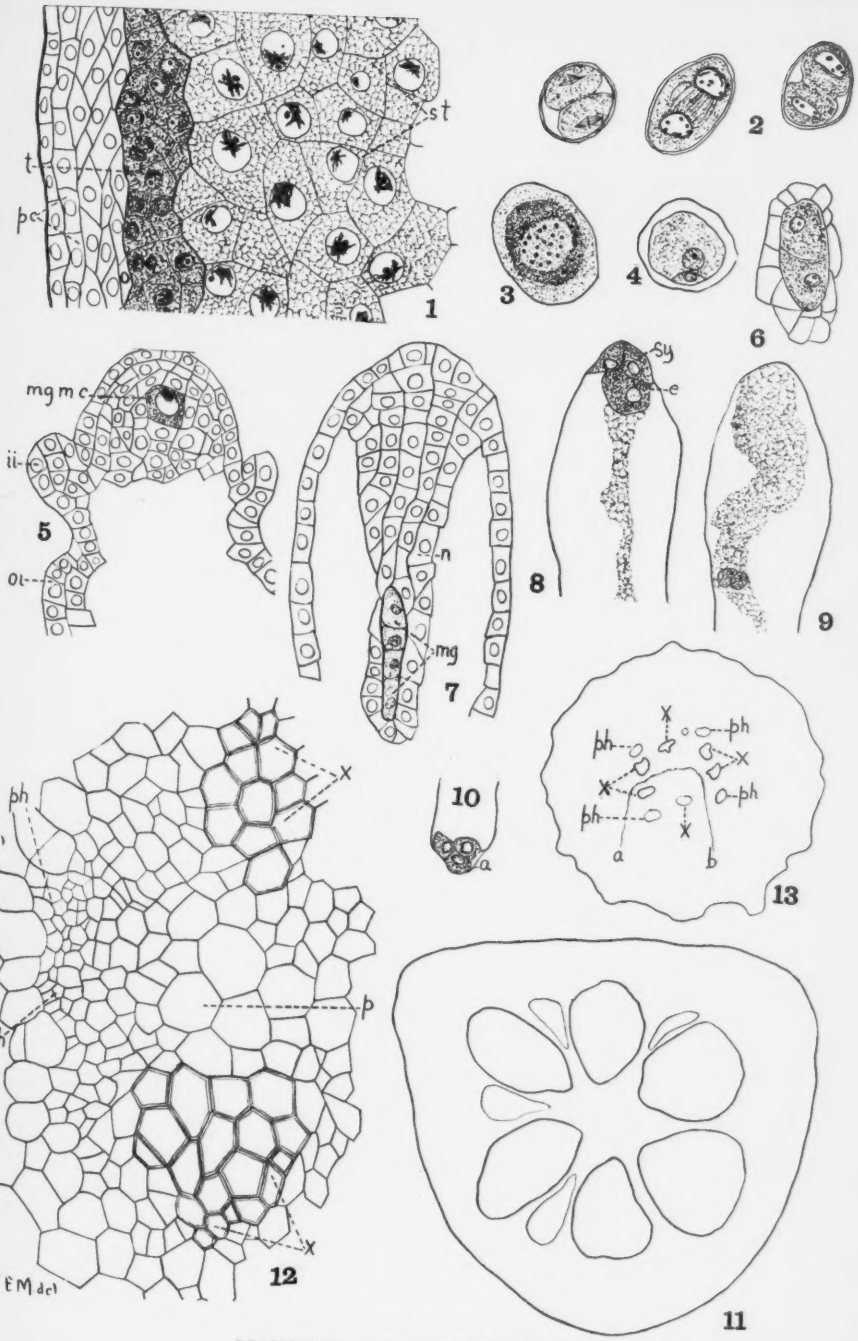
FIG. 12.—Transverse section of portion of central region of diarch root; ×590.

FIG. 13.—Transverse section of hypocotyl; ×50.

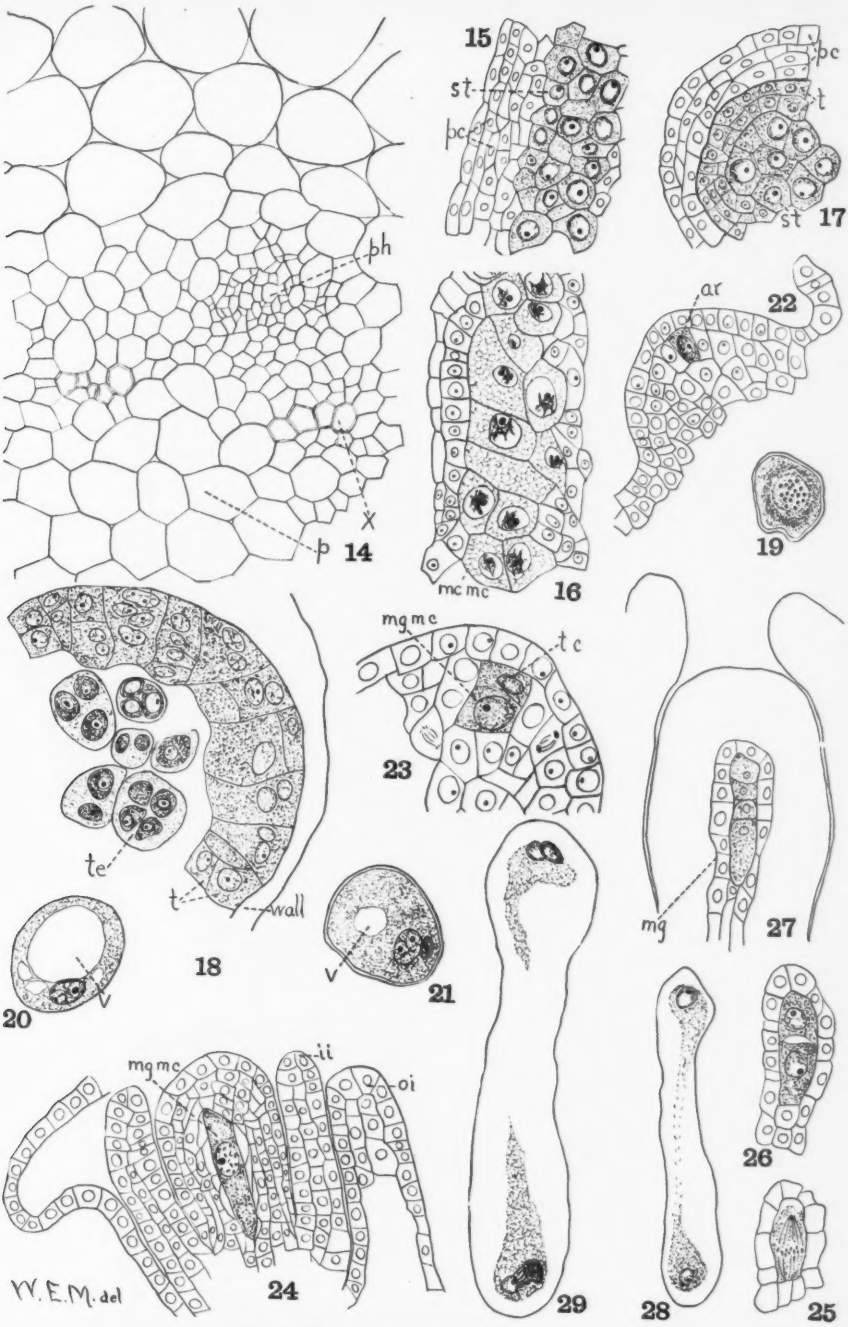
FIG. 14.—Transverse section showing details of the portion of fig. 13 indicated by line *ab;* ×300.

Magnolia

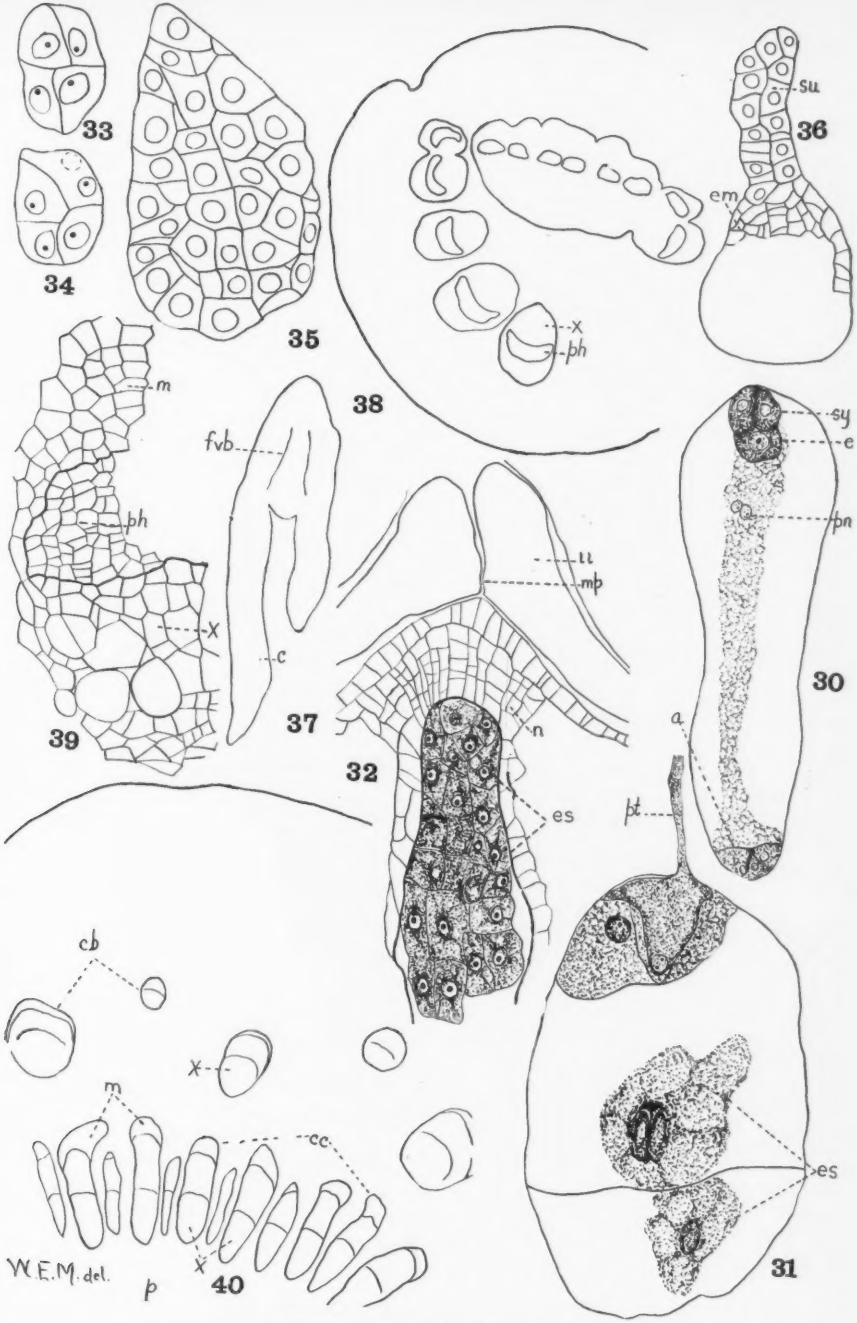
FIG. 15.—Longitudinal section of part of anther; sporogenous tissue differentiated; ×350.



MANEVAL on MAGNOLIACEAE



MANEVAL on MAGNOLIACEAE



MANEVAL on MAGNOLIACEAE

FIG. 16.—Longitudinal section of part of anther; microspore mother cells in synapsis; ×300.

FIG. 17.—Transverse section of anther; tapetum and sporogenous tissue differentiated; ×350.

FIG. 18.—Transverse section of anther; tetrads of microspores; ×300.

FIG. 19.—Section of pollen grain with reduced number of chromosomes; ×500.

FIG. 20.—Uninucleate pollen grain; ×350.

FIG. 21.—Binucleate pollen grain; ×350.

FIG. 22.—Longitudinal section of ovule with archesporial cell; ×300.

FIG. 23.—Longitudinal section of ovule through megaspore mother cell and tapetal cell; ×500.

FIG. 24.—Longitudinal section of ovule; megaspore mother cell; integuments; ×300.

FIG. 25.—First division of megaspore mother cell; ×350.

FIG. 26.—Megaspore mother cell divided; ×350.

FIG. 27.—Tetrad of megaspores; ×350.

FIG. 28.—Longitudinal section through binucleate embryo sac; ×350.

FIG. 29.—Longitudinal section through tetranucleate embryo sac; ×350.

FIG. 30.—Longitudinal section through mature embryo sac; ×350.

FIG. 31.—Micropylar end of embryo sac; pollen tube; two-celled endosperm; ×600.

FIG. 32.—Longitudinal section of micropylar end of embryo sac showing early condition of endosperm; ×300.

FIG. 33.—Section of four-celled embryo; ×500.

FIG. 34.—Section of eight-celled embryo; ×500.

FIG. 35.—Longitudinal section through an older embryo; ×500.

FIG. 36.—Longitudinal section of embryo and suspensor; ×300.

FIG. 37.—Longitudinal section of embryo from mature seed; ×50.

FIG. 38.—Transverse section of petiole; ×50.

FIG. 39.—Transverse section of portion of a fibrovascular bundle of a petiole; ×300.

FIG. 40.—Transverse section of portion of peduncle; ×50.

THE MATURATION PHASES IN SMILAX HERBACEA

MARION G. ELKINS

(WITH PLATES IV–VI)

The material for this paper was collected in the spring of 1909 in the vicinity of New Haven, Connecticut. Both staminate and pistillate flowers were obtained with a view to studying nuclear conditions in both sexes. Staminate flowers, gathered on May 14, supplied nearly all the stages desired, as the flower buds in each inflorescence exhibited varying degrees of development. The pistillate flowers, maturing more slowly, were fixed May 26 and June 2. The series obtained from this material was very incomplete, only a few stages of the prophase of the heterotypic division being procured. Traces of the megaspores were visible in some flowers, but in most cases their location was represented by a dark, irregular line which suggested crushing or imperfect fixation.

Various killing fluids were used, but only two proved of any value, namely Flemming's fluid (weaker solution) and Juel's fluid. Sections were cut 6μ in thickness and stained with Flemming's triple stain or Haidenhain's iron haematoxylin.

The maturation phases in the microsporangium

The earliest observations of the sporogenous tissue were made after the telophase of the last vegetative mitosis and before the differentiation of the tapetum. Excluding the outer layer of cells in this tissue, which eventually become tapetal, the remaining cells are virtually pollen mother cells, and after a slight increase in size are ready for the phenomena characterizing meiosis. The nuclei of the young spore mother cells show small chromatin bodies or granules of variable size scattered through the finely granular linin meshes. A distinct reticulum is not present. Often the chromatin bodies may be seen in pairs or groups of four, but their distribution is generally irregular and does not warrant a conclusion that pairing is their typical arrangement.

A multinucleolate condition is typical of the nuclei of these cells. The nucleoli are variable in number and size and often somewhat angular in outline; several small bodies appear attached to the nucleoli (figs. 2, 3), which resemble papillae and will be so designated during the following description. As late as diakinesis nucleoli have been observed with one or more of these papillae. In the material prepared with the triple stain the nucleoli of the heterotypic prophase show one or more glistening white spots; these were at first considered to be vacuoles, but there is also the possibility that they represent papillae viewed on the upper surface of the nucleoli. The microspore mother cell in the early prophase is sometimes uninucleolate, though more often provided with two large nucleoli. However, at synapsis there is never more than one large nucleolus, which is no longer angular and is usually provided with a single papilla.

In connection with the study of the microspore mother cells of the early prophase, observations were made on the somatic nuclei of the nucellus. The appearance of the chromatin bodies and the nucleoli in such a nucleus (fig. 27) agrees very closely with that given for the nuclei of the young spore mother cells.

The author believes the uninucleolate condition (fig. 5) to be the result of union of the nucleolar elements. Fig. 1 shows two nucleoli connected by a short, deeply stained strand, while fig. 2 shows two nucleoli in a later stage of fusion. The papillae described above are probably nucleolar fragments or very small nucleoli which are in the process of fusing with the larger nucleoli. Fusion of all the nucleolar matter apparently does not take place; as late as diakinesis small globular bodies are often found which are distinct from both the nucleolus and the chromosomes. The papillate condition of the nucleolus also persists until the nucleolus disappears at the metaphase.

GATES (14) describes parallel phenomena in the sporogenous cells of *Oenothera rubrinervis*. Here the nucleus is provided with one large nucleolus accompanied almost invariably by smaller nucleolar bodies. Fusion of these bodies takes place; the number present in later stages depends on the amount of fusion. This seems to vary. One large nucleolus is always present until the

disappearance of the nuclear membrane; there are also one or two small nucleolar bodies which remain through the metaphase of the heterotypic division and are sometimes observed on the homotypic spindle.

This papillate appearance of the nucleolus may be interpreted in quite another way, namely, as a process of chromatin budding from the nucleolus. CARDIFF (5) figures nucleoli with similar papillae in young spore mother cells, and suggests that a papilla may represent the beginning of a chromatin thread formed from the nucleolus. Miss NICHOLS (32), in a study of several species of *Sarracenia*, concludes that the nucleolus of the pollen mother cells elaborates the chromatin, and figures nucleoli with small bodies attached, which represent the chromatin emerging from the nucleolus. DARLING (7) describes the budding of chromosomes from the nucleolus in the prophase of the heterotypic division in *Acer Negundo*. In the somatic nuclei of the root tip of *Phaseolus*, WAGER (43) describes the nucleolus as being connected with suspending fibers along which the chromatin from the nucleolus passes. These fibers become much thickened with the accumulation of chromatin and finally break up into chromosomes. With the loss of chromatin the nucleolus shrinks, becomes detached from the chromosomes at the metaphase, and finally disappears. The conclusions of WAGER, however, are disputed by MARTINS MANO (26), who made a similar study of *Phaseolus* and concluded that the chromosomes are not of nucleolar origin. He advances this interpretation: at the telophase certain portions of the chromosomes are drawn out into threads which anastomose and form a chromatic reticulum. In the following prophase the reticulum gradually assumes the form of bands with connecting fibers; the bands contract and ultimately break up into chromosomes. The appearance that WAGER describes as "suspending fibers" MARTINS MANO interprets in another way. The nucleolus is formed independently from nucleolar substances, but in close contact with chromatin elements. When the perinucleolar vacuole is formed, there is a consequent repulsion of the nuclear reticulum; certain parts of it remain attached to the nucleolus by reason of the elasticity and viscosity of the chromosomes. If the nucleolus furnishes

any substance to the chromosomes it is not done by means of "suspending fibers."

Fig. 3 seems to show a condition like that described by DARLING and WAGER. There is a great similarity between the papillae attached to the nucleolus and the chromatin bodies lying free in the linin network. On the other hand, the presence of numerous darkly staining granules in the sporogenous and somatic nuclei and the presence of a nucleolus as late as diakinesis argue against the resolution of the nucleolus into chromatin bodies.

SYNAPSIS.—The term "synapsis" has been defined in two ways. Most botanists use the word to denote the period of maximum contraction of the chromatic elements in the prophase of the heterotypic division. Zoologists call this stage "synizesis" (MCCLUNG **25,** JORDAN **20**) and assign the name "synapsis" to the period of approximation of parental chromosomes. GRÉGOIRE (**17**) defines synapsis as covering stages from leptonema to strepsinema. In this paper "synapsis" will be considered as synonymous with "synizesis."

The presynaptic phases in *Smilax herbacea* are apparently simple; the linin mesh contracts (fig. 6), drawing the chromatin bodies together into an increasingly close proximity. As there is no chromatic reticulum or pairing of thin filaments, the process resolves into the mutual approach of chromatic bodies. MIYAKE'S (**28**) description of chromatin behavior in *Lilium* corresponds closely to this account. During synapsis (fig. 7) the nucleolus is almost invariably at one side and projecting from the synaptic mass; delicate linin threads bridge the karyolymph and connect the chromatic ball with the nuclear membrane. For a time the granular nature of the chromatin is maintained; toward the end of synapsis, however, the chromatin becomes arranged in a much interwoven beaded filament (fig. 8).

Views in regard to synapsis and its importance range from those that discard it as of no significance or due to faulty technique (SCHAFFNER **37**) to those that assign to it the function of bringing together parental elements in closest union and mutual influence. LAWSON (**22**) denies the presence of any real contraction and states that the phase known as synapsis is due to an enlargement of the

nuclear cavity, the chromatic mass remaining the same size. In 1899 GUIGNARD (**19**) reported the absence of synapsis in *Naias major*. CARDIFF (**5**) has described this phase as the deferred culmination of fertilization. The later tendency is to admit its presence as a normal stage and to consider it only a time of great shortening and thickening of chromatic filaments (GRÉGOIRE **17**, BERGHS **2**, DAVIS **8**). GRÉGOIRE in his discussion of synapsis admits that it is not a universal phenomenon. When it does occur, he believes it can have no rôle to play in the process of reduction, but is itself a result of certain nuclear activities. He further suggests that the appearance of synapsis may be emphasized by the growth of the nuclear cavity or by an artificial contraction caused by fixing reagents enhancing the natural contraction. GATES (**16**) states that it is evident many changes take place during synapsis, though there may be no interchange or influence between homologous chromosomes. He points out (**15**) that such influences may take place at any time during the sporophytic phase of the life cycle. ERNST (**12**) considers synapsis normal, otherwise a similar sensitiveness to fixing fluids ought to show in vegetative mitosis of corresponding stages. There is one possibility favoring artifact, namely, that the progressive stages of mitosis may be accompanied by chemical changes in the chromatic substance which cause different reactions to fixing fluids. It is difficult, however, to conceive of a chemical change occurring in a heterotypic prophase which would not also occur in a somatic prophase. Moreover, there is no experimental basis for this view, though NĚMEC (**30**) by microchemical tests demonstrated differences in the chromatin of resting and dividing nuclei.

The contraction of the nuclear contents is very striking in *Smilax herbacea* (cf. figs. 5, 6, 7), and is without doubt a normal condition. It is difficult to assign synapsis a rôle in *Smilax*. It is evident that the appearance of the nucleus after synapsis differs markedly from the preceding conditions; the chromatin elements pass into synapsis as distinct bodies and emerge in a homogeneous filament. Until more light is thrown upon this phase, we can only vaguely state that synapsis may facilitate the proper placing of the paired parental elements in the chromosomes and the chromo-

somes in the spireme. The chromosomes in *Smilax herbacea* never appear as definite units until the segmentation of the spireme.

POSTSYNAPSIS.—The much coiled filament of late synapsis emerges as a fairly thick thread slightly beaded (fig. 8), which later assumes a homogeneous character. The double nature of the spireme is early discernible (fig. 9). With the continued loosening of the knot the split is sometimes obliterated, but the spireme as it is distributed throughout the nuclear cavity is distinctly double; this separation of previously paired[1] chromatic elements does not appear simultaneously in all parts of the spireme (figs. 10, 11, 12). Shortening and thickening of the spireme proceeds as usual, followed by a sort of semi-segmentation of the double filament. At intervals along the spireme occur places where each longitudinal half is apparently constricted to a delicate thread (fig. 13). This appearance is due doubtless to incomplete transverse segmentation with subsequent pulling apart of the double segments; the attached portions thus are drawn out into fine threads. The bivalent chromosomes resulting from the completion of the transverse division of the spireme are long and slightly twisted about each other (figs. 14, 14*a*). Though not a typical strepsinema, this condition corresponds to strepsinema as described by GRÉGOIRE (**17**).

The shortening and thickening process continues, resulting in the characteristic diakinetic gemini, the univalent halves of which lie, indiscriminately (fig. 15), parallel, at right angles to each other, or in the form of V's or X's. Traces of linin threads can be seen attached to the ends of the chromosomes. The nucleolus is present at this stage (though not figured), but disappears before the metaphase. Small globular bodies scattered about among the gemini appear in many of the nuclei in addition to the nucleolus. These are probably small nucleolar bodies as previously described.

METAPHASE.—Many of the gemini retain the semblance of a V on the spindle (fig. 16), though occasionally the homologous chromosomes are oriented in a straight line. Intermediate stages occur between these two types of orientation. In many cases the chromosomes show a distinct splitting while at the equator; this is more marked as the chromosomes pass toward the poles, though

[1] Previous pairing is assumed to have taken place.

it does not become a complete fission (fig. 19). This is without doubt a genuine splitting preparatory for the homotypic mitosis.

As the first division is merely a separation of chromosomes, the true mitosis being deferred until the second division, it is not surprising that the fully formed chromosomes exhibit a tendency to fission, the normal consequence of a mature condition in meristematic cells, long before actual mitosis is permitted to take place. FARMER (**13**) expressed a similar idea when he said "with the inception of karyokinetic activity the spireme thread undergoes the longitudinal fission characteristic of ordinary somatic division, although the actual separation of these longitudinal halves is deferred until the next mitosis."

The separation of the chromosomes at the equator and their passage to the poles takes place in the usual manner. Frequently the chromosomes of one or more pairs separate and move away from the equator earlier than the majority (figs. 17, 18).

INTERKINESIS.—At the telophase the chromatic elements appear in the form of a spireme which is disposed about the periphery of the newly formed nuclear membrane. The daughter nuclei are usually elliptical, though sometimes they are slightly curved, presenting a concave surface toward the equatorial plate. MIYAKE (**28**) finds in *Lilium Martagon* a partial formation of a thread, but usually there is little change in the form of the chromosomes during interkinesis. In *Smilax herbacea* the split which was observed in the metaphase and anaphase, homotypic in nature, is sometimes faintly discernible, but usually lost to view. Vacuolation of the chromatin band, if it may be said to occur at all, is very slight. In fact, the transitory character of this phase does not call for extensive alveolization. We have here in reality a prophase of the homotypic division. GRÉGOIRE (**17**) describes the heterotypic division as a process intercalated in the prophase of the homotypic division.

Conditions reported during interkinesis vary in different plants. In *Oenothera gigas*, according to DAVIS (**9**), the daughter chromosomes maintain their form and distinctly show the homotypic split, thus giving an appearance similar to diakinesis. GATES (**16**), describing the same species, states that some of the chromosomes

pass through interkinesis in a compact condition, while others become vacuolate. In *Nephrodium molle* (YAMANOUCHI **45**) the chromosomes become vacuolate, but their identity is not lost. ALLEN (**1**) cites the formation of a spireme during the telophase of *Lilium canadense*. In *Pinus* and *Thuja* (LEWIS **24**) the identity of the chromosome is completely lost. NICHOLS (**31**) reports a similar condition in *Juniperus*.

HOMOTYPIC DIVISION.—In preparation for the second division the nuclear membrane disappears[2] and is succeeded by the formation of a spindle whose axis corresponds with the greater axis of the daughter nucleus. The daughter spireme is at first spread out on the spindle from pole to pole (fig. 21); later the chromatic mass contracts (fig. 22) and occupies a position at the equator of the spindle. At this time the spireme seems to be resolving into chromosomes (fig. 22). Throughout these stages there is no sign of a double filament; in fact the whole structure is indistinct. Figs. 23 and 24 show fully formed chromosomes which have split into daughter chromosomes. A side view of the spindle (figs. 23, 24) presents chromosomes apparently shaped like dumb-bells. A comparison of the above mentioned figures with fig. 25, a polar view of the equatorial plate, explains the actual condition. The daughter chromosomes are paired in the form of V's; the open ends of the V's are turned outward, the arms of the V's are nearly at right angles to the axis of the spindle. The appearance of the chromosomes of figs. 23 and 24, as described above, is due to the fact that only the tips of the chromosomes at the open ends of the V's can be seen; the seeming connection between the chromosome tips is occasioned by an indistinct view of the apices of the V's.

The separation of the daughter chromosomes as in the first division is not simultaneous. Fig. 23 shows a chromosome well on its way toward one pole before its sister chromosome, or the

[2] LAWSON in a recent paper (**23**) claims that the nuclear membrane does not break down or collapse as was formerly supposed to be the case. On the contrary, it behaves as a semi-permeable plasmatic membrane. Changes in the quantity and form of the chromatin previous to the metaphase are apparently accompanied by a change in the osmotic relations of the karyolymph. As a result of this there is a gradual decrease in the volume of the nuclear vacuole until the nuclear membrane closes in about the chromosomes; each chromosome becomes a single osmotic system.

other chromosomes, have moved far from the equatorial plate. Fig. 26 also shows chromosomes in the anaphase lagging behind at the equator, while the majority have nearly reached the poles.

CHROMOSOME NUMBER.—The metaphase of the second division represents the most favorable opportunity for the chromosome count. The chromosome number, however, was not determined with any finality. In diakinesis the chromosomes, though few in number, are so large that they obscure each other. A comparison of the counts attempted during diakinesis and the second metaphase places the haploid number of chromosomes at either 12 or 13.

Postsynapsis in the megasporangium

The difficulty of obtaining maturation stages of the megaspore mother cell discouraged a study of meiosis in the pistillate material. Of the many slides prepared, the majority showed synapsis; in addition only a few postsynaptic stages were procured. The nuclei of the megaspore mother cells are larger than those of the microspore mother cells, and when desirable stages are found they are exceedingly favorable for study.

The first stage noted after synapsis represents the nucleus as containing a mass of loosely interwoven filaments undivided and slightly beaded (fig. 28). The filaments thicken (fig. 29) and split longitudinally (fig. 30). Before the spireme breaks up into the bivalent chromosomes, it passes into strepsinema; the halves of the double filament draw apart, twist about, and cross each other (fig. 31). Attenuated portions of the spireme may be observed; transverse segmentation has begun and isolated pairs of chromosomes may be seen near the periphery of the nucleus.

In diakinesis the paired chromosomes occupy many positions with respect to each other, seldom lying strictly parallel. In fact, the description of diakinesis in the staminate loculus applies here. In fig. 33 several of the chromosomes offer a trace of a split, probably a precocious homotypic fission. In the microspore mother cells this split was not observed until the metaphase. This homotypic fission is described as occurring earlier in plants studied by the metasyndetists (MOTTIER, LEWIS, FARMER, DAVIS,

and others). MOTTIER (**29**) finds gemini in *Tradescantia* having double paired segments. This doubling is supposedly due to the reappearance of a split in the spireme which is believed to be a genuine homotypic fission and hence comparable to the split found in the paired segments of gemini in *Smilax*.

Mode of reduction

The mode of reduction in *Smilax herbacea* is essentially parasyndetic, though the procedure in the prophase seems to depart from the method described by GRÉGOIRE (**17**) for parasyndesis. According to GRÉGOIRE, the chromatin in the prophase takes the form of thin paired filaments (leptonema) which fuse (zygonema), shorten and thicken (pachynema), and again separate (strepsinema). In *Smilax* the chromatin in the prophase is distributed in granules, which are frequently seen in pairs. That which takes place between this condition and the spireme is obscured by synapsis. The construction and relative arrangement of the chromosomes in the spireme can be inferred only from subsequent behavior. After synapsis, two longitudinal splits occur; the first appears early in the spireme and can be traced through strepsinema to diakinesis; the second split shows sometimes in the univalent halves of the gemini at diakinesis, but more often not until the metaphase. From this we may infer that the first doubling is a separation of previously paired elements and that the chromosomes or chromatic bodies are placed side by side in the spireme. The second doubling is a genuine fission.

Discussion

PERSISTENCE OF CHROMOSOMES.—Much of the cytological work of recent years has brought forward directly or indirectly the question of the persistence of chromosomes or of some smaller unit. The rediscovery of the work of MENDEL has given added impetus to the hope of finding a physical basis for heredity or the unit characters of MENDEL which, according to experiment, seem to pass from generation to generation inviolate. Opinions concerning the existence of this cytological unit necessarily vary. Some favor the idea of persistent chromosomes; some maintain that

the unit is smaller; while the observations of others imply the non-existence of a persistent chromatin body.

The work of OVERTON strongly supports the theory of chromosome persistence. In the resting somatic nuclei of *Thalictrum purpurascens* and *Calycanthus floridus* the chromosomes are represented by definite visible bodies, the prochromosomes (OVERTON **33**, **34**). From his study of the nuclei in the pollen mother cells of *Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, and *Calycanthus floridus*, he draws the conclusion that the chromosomes never lose their identity in either somatic or germ nuclei. Even on the spireme the chromosome unit is distinctly visible. During interkineses (OVERTON **35**) of somatic mitoses progressive vacuolation and enlargement of the chromosomes take place, but the chromosome outline can always be traced. LAIBACH (**21**) in working on the Cruciferae finds that the chromosomes remain as clearly defined in the resting condition as during mitosis.

ROSENBERG (**36**), in the resting nucleus of the hybrid *Drosera longifolia*×*rotundifolia*, finds paired chromatic bodies that equal the number of somatic chromosomes. These he calls prochromosomes or centers of chromosome formation. DAVIS (**9**) described chromatic bodies in the nuclei shortly after the last division in the archesporium of *Oenothera gigas*, which he thinks probably are chromatin centers or prochromosomes.

On the other hand, the theory of nucleolar origin of chromosomes does not support the view of chromosome permanence. The author has already referred to the work of WAGER (**43**) and DARLING (**7**) describing the budding of chromosomes from the nucleolus. SHEPPARD (**38**) investigated the behavior of the nucleolus in *Hyacinthus*. In the spireme stage he found the nucleolus apparently being drawn out upon the chromatin threads by means of nucleolar pseudopodia connected with the chromatin threads. Here, as described, the chromatin does not originate entirely from the nucleolus. BERGHS (**3**) found large nucleoli in the somatic cells of *Spirogyra* which break up into bodies partly chromatic and partly achromatic equal to the number of chromosomes. A more detailed citation of this will occur later. Miss DIGBY (**11**) states that there is no relation between the number of

chromatic aggregations in the resting nucleus of *Galtonia candicans* and the number of chromosomes; moreover, in the telophase of the somatic divisions the chromosomes lose their identity, their centers dissolving and the chromosomes breaking into small portions.

Many cytologists compromise on a middle ground and assume that bodies which are smaller than the chromosomes and into which the chromosome is divisible, are the chromatic units. These have been styled as pangens (MOTTIER **29**) or chromomeres (ALLEN **1**, LEWIS **24**). In the ordinary use of the above terms the pangen represents a smaller unit than the chromomere; in this connection the terms are used simply to designate a small chromatic body of no determined size. The chromosome represents a definite group of these units and is probably formed for the purpose of facilitating segregation and mitosis. ALLEN figures the actual union of chromatin granules in the spireme, with their subsequent separation. MOTTIER finds no evidence of prochromosomes but supports the theory of the individuality of pangens. LEWIS describes granules in the resting nucleus in excess of the number of chromosomes.

The differences among the above citations are not as serious as they might seem. By the adoption of a hypothetical unit smaller than the chromosome, it is not difficult to imagine that its appearance, whether alone or in close approximation to its fellows, might vary and vary much with the different species of plants studied. In the plants studied by OVERTON and LAIBACH the chromosomes may be said to pass from one stage to another always in definite uniform groups, the prochromosomes. We may say these bodies maintain their permanence because of an unchanging mutual attraction of the chromomeres in each chromosome. We may conceive of another condition in which the mutual attraction of the chromomeres in each chromosome group varies with the resting and active stages of the nucleus. Although ROSENBERG (**36**) found the somatic number of chromatin bodies in the resting nuclei of the hybrid *Drosera*, he considered them as chromatin centers about which chromatin units congregated in the prophase, always with the same relative arrangement. This theory advanced by ROSENBERG may be modified and extended to

cover a condition where no chromatin centers are visible, but in which the chromatin units, or small groups of units, arising from the fragmentation of a single chromosome, exert a mutual attraction and come together in a uniform body during the prophases.

It is somewhat more difficult to apply this conception to the cases of nucleolar origin of chromosomes. Although little is known about the nature and structure of the nucleolus, it seems plausible that the same relations between chromomeres may exist whether they are inclosed in a comparatively small body, the nucleolus, grouped in several small bodies, the chromosomes, or scattered in the nucleus.

An obstacle to the view of the persistence of either chromosomes or smaller chromatin units arises, however, when we consider the recent work of certain authors, such as that of Miss DIGBY (**10**) on *Galtonia candicans*, or that of GATES (**16**) on *Oenothera gigas*. Miss DIGBY describes a condition in *Galtonia* in which chromatin buds off from the nuclear framework, synaptic knot, or nucleolus, and passes into the cytoplasm or even into neighboring cells; these buds eventually disintegrate. Though Miss DIGBY implies that the parent nuclei develop normally, she does not describe their development beyond the spireme stage. However, she cites cases where entire loculi contained aborted pollen mother cells. GATES describes a similar phenomenon in *Oenothera gigas*. During the synaptic stage there is an extrusion of a part of the chromatic matter of the spireme into an adjoining cell; the extruded portion degenerates, but the nucleus from which it came behaves normally. CARRUTHERS (**6**), in a description of the cytology of *Helvella crispa*, states that there are extrusions of chromatin-like material from the poles of the nucleus, which disintegrate in the cytoplasm and take up nucleolar stains. GRIGGS (**18**) says, of the masses of chromatin in the nuclei of *Rhodochytrium*, a portion remains free and is cast into the cytoplasm or remains as beads on the spindle fibers, while the rest of the chromatin forms the chromosomes.

It is also not without interest to recall the condition of *Spirogyra* (BERGHS **3**, MITZKEWITSCH **27**) where a chromatic nucleolus during the anaphase shows tiny chromosomes enveloped in its mass; at the metaphase the nucleolus is divided and the halves move

toward the poles of the spindle where the nucleolar mass is resolved into large chromosomes. Upon decolorization, small portions, the size of the prophasic chromosomes, at the equatorial ends of the large chromosomes strongly retain the stain. The deeply stained portions of the large chromosomes BERGHS considers the "chromosomes veritables." In describing the larger bodies the word "chromosome" is used merely for convenience.

From the observations just noted, we may draw the conclusion that there are substances which are not chromatic in the sense of being the carriers of hereditary qualities, but which at some stages have the same appearance and pass through certain phases in closest proximity to the real chromosome. CARRUTHERS (**6**) suggests that the extruded bodies are masses of nutritive material for which the nucleus has no further use.

Conditions in *Smilax herbacea* do not support the theory of persistence of the chromosome as a physical unit, but of a smaller unit. The number of chromatin bodies in the microspore mother cell exceeds the number of somatic chromosomes; the same is true of the somatic nuclei of the nucellus. As these chromatin bodies vary in size, we consider that they are aggregates of units or chromomeres; also that the size of the chromomere aggregate varies with the number of units contained in it. Finally, during synapsis the chromomere aggregates form chromosomes according to a law of natural affinity. There is no evidence of a loss of chromatin or chromatin-like material.

PAIRING OF CHROMATIC ELEMENTS.—Closely connected with the theory of the persistence of chromatic units is the theory of the pairing of parental elements throughout the sporophytic phase. Before the theory of permanent, paired chromatic units was advanced, "pairing" was described only in connection with the prophase of the first meiotic division where parallel conjugation (parasynapsis or parasyndesis) of thin chromatic filaments was considered typical. A large group of cytologists now present a different mode of conjugation, namely, of the "end to end" type (telosynapsis or metasyndesis).

The difference between the two methods is not as important as it seems to be. Too much stress has been laid on the comparative

merits of parasyndesis and metasyndesis. Granted that the chromosomes are fully formed, so far as the arrangement of constituent parts is concerned, at the time of synapsis, there need be no difference in the ultimate result whether the homologous chromosomes appear in the spireme side by side or one ahead of the other; in either case the paired chromosomes are adjacent and are not prevented by their previous arrangement from exhibiting the same relations from diakinesis through succeeding stages. JORDAN (20) suggests that both parasynapsis and telosynapsis may occur in the same prophase; that is to say, the "end to end" arrangement of chromosomes in the spireme is frequently followed by a pronounced loop formation, resulting in a parallel approximation of chromosomes. On the other hand, parasynapsis may be followed by fusion of the ends of paired chromosomes (diakinesis). GATES (15) explains the appearance of both types of conjugation from a mechanical standpoint. According to his view short chromosomes are particularly adapted to telosynapsis, while long chromosomes are parasynaptic.

The extension of the theory of chromosome pairing to cover the entire sporophytic phase is supported by the observations of several investigators. STRASBURGER (39), in a study of the root tips of *Pisum*, found many cases where the chromosomes were grouped in pairs on the nuclear plate. He concludes that the parental chromosomes in the nuclei of the sporophyte generation do not form two separate groups, but that the homologous chromosomes occur in definite positions with respect to each other. He also figures a similar condition in an integument cell of the ovule of *Lilium Martagon* (STRASBURGER 40). Miss SYKES (42) describes a paired arrangement of chromatic elements in the somatic nuclei of *Hydrocharis Morsus-ranae* and *Bryonia dioica*. *Lychnis dioica* and *Sagittaria montevidensis* show fully formed chromosomes lying in pairs. OVERTON (34) states that, in the somatic nuclei of plants which he has studied, definite chromatic bodies were visible lying in pairs (*Campanula grandis, Helleborus foetidus, Thalictrum purpurascens, Calycanthus floridus*). BONNET (4), however, finds no satisfactory evidence of pairing in the diploid nuclei of the *Yucca*, although he finds definite chromosomes.

In plants which do not possess the persistent chromosome, the chance of observing the paired arrangement of smaller groups of chromatic units is much lessened. As mentioned early in this paper, the chromomeres or chromomere aggregates in either somatic or germ nuclei of *Smilax herbacea* frequently appear in pairs or double pairs. This seems to occur too often to be merely a coincidence, yet it could not be determined as a universal condition.

THE SEX DETERMINANT IN PLANTS.—Although no reference to the sex determinant has been made in the preceding pages, this study was first attempted with the hope of finding the idiochromosome in a dioecious plant. WILSON (**44**), in his studies of the determination of sex in insects, places the decisive sex factor in the sperm. Here he found one-half of the sperms each carrying one or more extra chromosomes or a chromosome unique in size. All cases which cannot be placed in the above groups he relegates to a group where there is no physical variation of the chromosomes in the sperm cells, but where one may presume a physiological variation.

Botanists have been unsuccessful in their efforts to find the idiochromosome. DARLING (**7**) in working on the sexual cells of *Acer Negundo* (staminate material) found that one daughter nucleus after the first division contained a secondary chromatin mass; after the second division, two of the granddaughter nuclei each contained one more chromatin mass than the other two. In the resting stage, however, all the nuclei looked alike. To these secondary masses DARLING attached a possible sexual significance. Miss SYKES (**42**) found the nuclei of both sexes of unisexual plants she had studied (*Hydrocharis Morsus-ranae*, *Bryonia dioica*, *Lychnis dioica*, *Mercurialis perennis*, *Sagittaria montevidensis*, *Cucurbita Pepo*) to be identical in the number and form of the chromosomes. STRASBURGER'S (**41**) efforts to find a structural basis for the determination of sex were rewarded with negative results. On the nuclear reduction plate of *Melandryum rubrum* he observed a pair of chromosomes much larger than the other gemini; this same condition appeared again on the homotypic spindle. Thus, one large chromosome was distributed to each of the tetraspores.

However, he found that, though the four pollen grains of each group usually agreed in size, there were groups in which two of the pollen grains were larger than the corresponding two. This possible indication of sex differentiation was destroyed when he found a like condition among the pollen grains of hermaphrodite plants (*Lychnis Flos-jovis*, *Silene fimbriata*).

In *Smilax herbacea* no trace of an idiochromosome was found. During the metaphase of the first division one frequently found a chromosome pair which anticipated the other gemini in separating and moving toward the poles (figs. 17, 18), but no differences were observed in the homotypic division or in the tetraspores. Moreover, precocious chromosome movement away from the equator during the heterotypic division has been reported by CARDIFF (5) in *Salomonia*, a hermaphrodite.

For the present, then, we may assume a physiological difference in the tetraspores of dioecious plants which has no physical manifestation.

Summary

1. The nuclei of the young microspore mother cells each contain several nucleoli of varying size. The nucleoli fuse during the prophase, forming one large nucleolus at synapsis. During the early prophase the nucleolus is provided with several "papillae." These doubtless represent small nucleolar bodies which also fuse with the larger nucleoli. The nucleolus usually has at least one papilla until its disappearance at the metaphase.

2. The chromatin in the young microspore mother cell occurs in the form of granules or chromomere aggregates (the chromomere is here considered a chromatic unit).

3. There is no presynaptic reticulum, leptonema, or zygonema. The chromatin granules are held in an indefinite linin mesh.

4. Synapsis is reached by a contraction of the linin-supporting structure drawing the chromatin granules together.

5. The chromatic elements emerge from synapsis in the form of a spireme which soon becomes double.

6. The spireme shortens and thickens. Transverse segmentation of the spireme results in the formation of long paired chromo-

somes which continue to shorten and thicken, producing the characteristic gemini of diakinesis.

7. The separation of homologous chromosomes at the metaphase proceeds as usual. At this stage the chromosomes frequently show a split preparatory for the second division.

8. At the telophase a nuclear membrane appears. During interkinesis the chromatin is in the form of a band, apparently wound about the periphery of the nucleus. The band seems to be split or slightly vacuolate.

9. With the formation of the spindle of the second division the nuclear membrane disappears and the chromatic band resolves into chromosomes.

10. At the homotypic metaphase the longitudinal halves of the chromosomes separate.

11. The method of reduction in *Smilax herbacea* essentially coincides with the "hétérohoméotypique" scheme of GRÉGOIRE.

12. The persistent chromatic body in *Smilax* is a smaller unit than the chromosome.

13. The pairing of chromatic bodies was observed in the prophase, but not as a universal phenomenon. The same condition was evident in the nuclei of the nucellus.

14. An effort to find a sex determinant in *Smilax* was futile.

The writer wishes to express her indebtedness to Professor A. W. EVANS for suggesting this study and for his helpful advice and criticism.

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EXPLANATION OF PLATES IV–VI

Microspore mother cells

FIGS. 1 and 2.—Young spore mother cell; nucleoli fusing; ×2400.

FIG. 3.—Same as fig. 1; nucleolar "papillae"; ×2400.

FIG. 4.—Young spore mother cell; paired chromatin bodies; ×2400.

FIG. 5.—Spore mother cell with one papillate nucleolus; chromatin bodies in pairs; ×2400.

FIG. 6.—Early synapsis; ×1725.

FIG. 7.—Synapsis; ×1725.

FIGS. 8, 9.—Late synapsis; ×1725.

FIG. 10.—Spireme; ×1725.

FIG. 11.—Early spireme stage; ×2400.

FIG. 12.—Spireme; ×2400.

FIG. 13.—Late spireme; beginning of transverse segmentation of spireme; ×2400.

FIG. 14.—Strepsinema; ×2400.

FIG. 14*a*.—Pair of homologous chromosomes; same stage as fig. 14; ×2400.

FIG. 15.—Diakinesis; ×2400.

FIGS. 16–18.—Metaphase; ×2400.

FIG. 19.—Late anaphase; ×2400.

FIG. 20.—Interkinesis; ×2400.

FIGS. 21–26.—Homotypic phases.

FIG. 21.—Prophase; ×1725.

FIG. 22.—Late prophase; ×1725.

FIG. 23.—Metaphase; ×1725.

FIG. 24.—Metaphase; ×1725.

FIG. 25.—Metaphase; polar view of equatorial plate; ×1725.

FIG. 26.—Late anaphase; ×2400.

FIG. 27.—Nucleus from somatic cell of young ovule; ×2400.

Megaspore mother cells

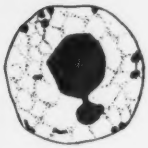
FIG. 28.—Early spireme stage; ×2400.

FIG. 29.—Spireme; ×2400.

FIG. 30.—Spireme; later stage than fig. 29; ×2400

FIG. 31.—Strepsinema; ×2400.

FIGS. 32, 33.—Diakinesis; ×2400.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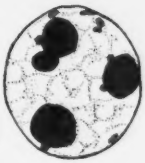
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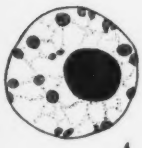
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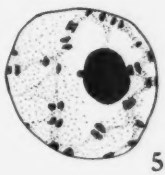
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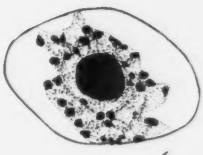
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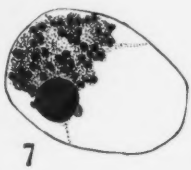
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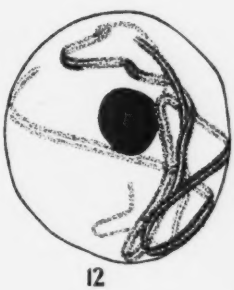
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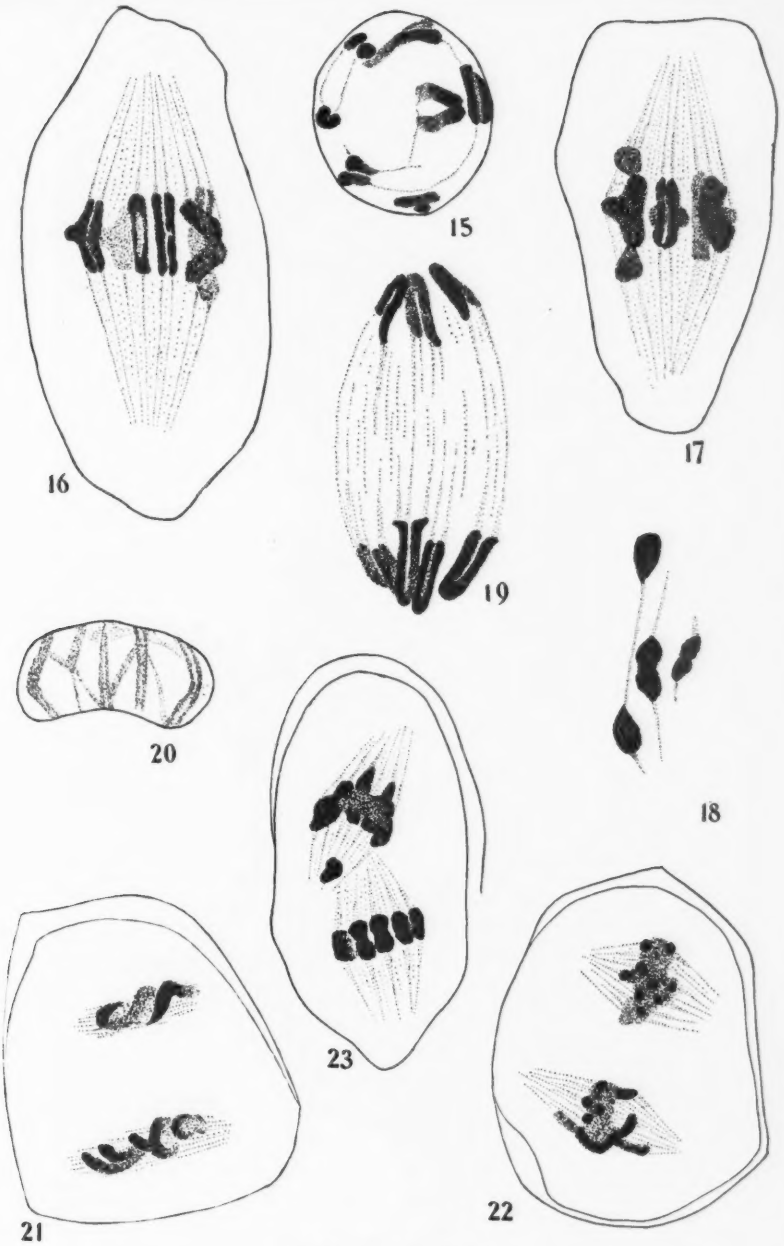
ELKINS on SMILAX



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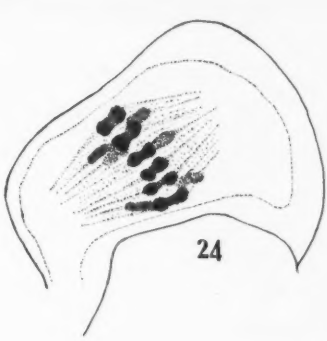

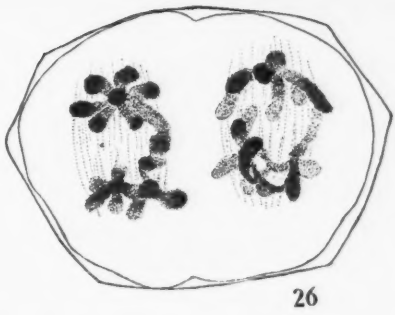

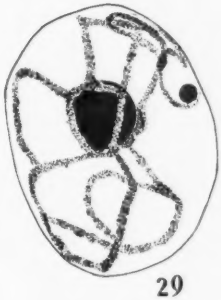

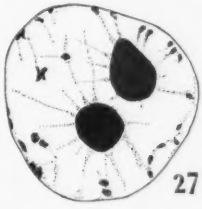

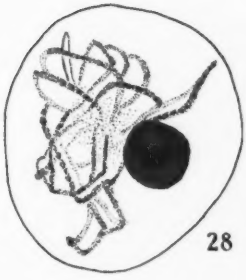

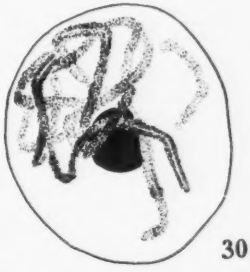

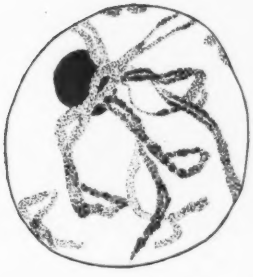

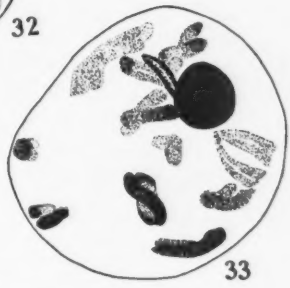

ELKINS on SMILAX

COMPARATIVE HISTOLOGY OF ALFALFA AND CLOVERS

KATE BARBER WINTON

(WITH EIGHT FIGURES)

Perennial leguminous forage plants are growing in importance both for green feeding and for hay, and some of them, notably alfalfa, red and alsike clovers, are well adapted for grinding into meal. The work detailed in this paper was undertaken to facilitate the microscopic identification of the species named in mixed cattle foods.

The highest feeding value of the hay or "meal" is obtained from plants cut in early flower, though the more or less mature fruits and seeds are not infrequently found in the products on the market, especially in alfalfa meal.

Alfalfa

Alfalfa (*Medica sativa* [L.] Mill., *Medicago sativa* L.) is a native of Asia and has been cultivated for fodder since long before the Christian era. It is now grown in both hemispheres, especially in the arid and semiarid regions of the Southwest, for use either fresh or dried. As the hay is brittle, resulting, when fed from the bale, in a considerable loss of leaves, the product is often kiln-dried and ground to a meal.

FIG. 1.—Alfalfa (*Medica setiva*): *I*, leaf, ×1; *II*, flower, ×3; *III*, seed, ×3; *IV*, fruit, ×3.

Ordinary alfalfa, or lucerne, branches profusely and bears alternate leaves (fig. 1, *I*) consisting of three distinct obovate to lanceolate leaflets finely dentate at the apex. The plant is described as glabrous; hairs, however, are evident under a lens and are highly characteristic with higher magnifications. The flowers (fig. 1, *II*) appear in racemes of 8–25 each and wither after flowering. They are of the distinctly papilionaceous type, small (8–10 mm. in length),

and delicate in structure. The hairy calyx consists of a tube and 5 teeth-like lobes of about the same length as the tube, at the base of which is inserted the violet-colored corolla. The 10 stamens are combined in two sets; the ovary is one-celled with several ovules. At maturity the brown pods (fig. 1, *IV*) are coiled 2–4 times in close spirals, the diameter of the coil being about 4 mm. The greenish-brown seeds (fig. 1, *III*), up to 3 mm. in length, are somewhat kidney-shaped.

Many varieties of alfalfa, less widely grown, vary in flower color, through blue, white, and green, to yellow, and in number of pod coils, seeds, and leaflets.

Histology

Stem (fig. 2).—The *epidermal cells* (*ep*) are several times longer than broad and arranged end to end in longitudinal rows interrupted frequently by stomata and their accompanying cells. The outer and inner walls are slightly thickened, the former having a cuticle with delicate striations evident in cleared preparations.

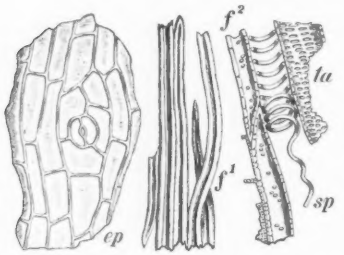

Fig. 2.—Alfalfa: elements of stem in surface view; *ep*, epidermis; f^1, bast fibers; f^2, wood fibers; *sp*, spiral vessels; *ta*, pitted vessels; ×160.

Bast.—Several layers of simple thin-walled chlorophyll-bearing parenchyma cells, interrupted occasionally, especially at the angles of the stem, by masses of collenchyma, form the outer tissues. Underlying this is a single layer of thin-walled crystal-bearing cells inclosing a zone of bast fiber bundles, each bundle being wedge-shaped in cross section. The individual fibers (f^1) are greatly elongated and have walls so strongly thickened that the lumen is often but a mere line.

Phloem.—This consists of a characterless mass of thin-walled cambium cells and sieve tubes.

Xylem.—The most evident elements of this woody tissue are the pitted (*ta*) and spiral (*sp*) vessels and the pitted wood fibers (f^2).

Pith.—This consists of comparatively large, thin-walled, pitted cells with no cell contents.

LEAF.—*Upper epidermis.*—The cells are approximately 35μ in diameter, although often elongated, especially over the veins. The cell walls are strikingly sinuous and of uniform thickness. Numerous simple stomata are scattered over the whole surface, and occasional hairs, similar to those so abundant on the lower surface, occur at the base of the leaf. Cuticular striations are very distinct in cleared material.

The *palisade layer* consists of very simple cells with breadth half the height.

Mesophyll.—The ground tissue of this layer is made up of a loose mass of parenchyma with no characteristic features; accompanying the simple bundles, however, are crystal-bearing cells (fig. 3, *cr*) of diagnostic importance. The latter are thin-walled and arranged more or less end to end in longitudinal rows. Each cell contains a single monoclinic crystal about 18μ in length, the facets of which often appear corroded.

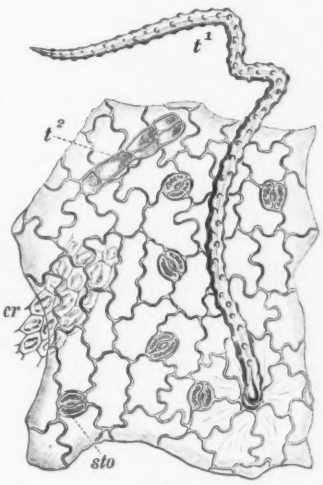

FIG. 3.—Alfalfa: lower epidermis of leaf with unicellular hair (t^1), capitate hair (t^2), and stoma (*sto*); *cr*, crystal cells accompanying bundles; ×160.

The *lower epidermis* (fig. 3) differs from the upper principally in the greater number of hairs which are scattered over the whole surface and margin of the leaf, being especially numerous along the veins. The cells surrounding the hair base form a rosette. The hairs are of two kinds, unicellular (numerous) and capitate (scattered). The unicellular hairs (t^1) are more or less sinuous, thick-walled, the lumen being a mere line, with small but prominent warts distributed over the entire length. They arise from a small, slightly thick-walled basal cell and average 800 μ in length and 15 μ in breadth, though the length varies up to over 1.5 mm. The

capitate hairs (l^2) consist of a stalk of two or three cells and a multicellular head, all the cells being thin-walled and frequently collapsed.

CALYX.—The *epidermis* bears unicellular and capitate hairs of the same general structure as those on the leaf. On the calyx tube the unicellular hairs are comparatively short and thick-walled, while on the lobes they are longer and thinner-walled, with correspondingly broader lumen. The simple bundle running out to the tip of each lobe is surrounded by a layer of crystal cells each containing a crystal averaging 18 μ in length.

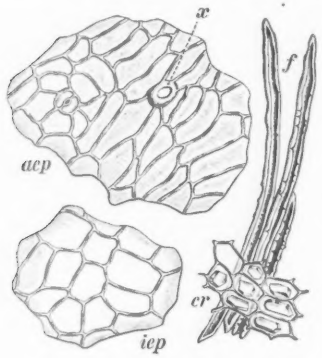

FIG. 4.—Alfalfa: elements of pod in surface view; *aep*, outer epidermis with hair scar (x); *iep*, inner epidermis; *cr*, crystal layer; *f*, fibers; ×160.

COROLLA.—The *epidermal cells* of the petals, at the base, are very thin-walled, elongated, and somewhat sinuous, and bear toward the tip papillae with striated cuticle. The bundles are very small, often but a single spiral vessel marking their course.

STAMENS.—The *filaments* consist of delicate cells similar to those of the petal in structure. The *anthers* have riblike thickenings over their whole surface.

PISTIL.—The *stigma* bears colorless papillae closely matted together.

The *style* is made up of small characterless cells except the outer half, which is covered with cells slightly thickened, apparently for mechanical support.

Ovary.—The small thin-walled epidermal cells bear numerous thin-walled unicellular and capitate hairs.

PERICARP (fig. 4).—The *epicarp* (*aep*) consists of a single layer of empty cells usually more or less elongated except at the stomata, about which they form a rosette. Hairs are frequently present, but often break off from the dried pod, leaving a scar (x) with a thickened wall.

Mesocarp.—The characteristic tissues are: crystal cells (*cr*)

with very thin walls, frequently side by side in rows, each cell containing a single crystal, and fibers (*f*) with rather blunt ends and pitted walls, the number of pits being most numerous in fibers with the thickest walls.

Endocarp (*iep*).—A single layer of epidermal cells without stomata completes the pericarp.

SPERMODERM (fig. 5, *S;* fig. 6).—The *palisade cells* (*pal*) are upward of 35 μ high and 8–10 μ broad, with rounded outer ends and a thin cuticle. A narrow light line (*l*), situated about 7 μ within the outer end, can be easily seen in cross section. As is

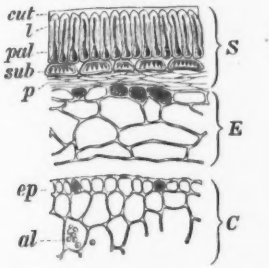

FIG. 5.—Alfalfa: seed in cross section; *S*, spermoderm consists of palisade cells (*pal*) with cuticle (*cut*) and light line (*l*), subepidermal layer (hour-glass cells) (*sub*), and parenchyma (*p*); *E*, endosperm; *C*, cotyledon with epidermis (*ep*) and aleurone grains (*al*); ×160.

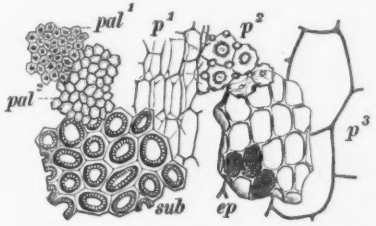

FIG. 6.—Alfalfa: elements of seed in surface view; *pal*¹, outer palisade cells; *pal*², inner palisade cells; *sub*, subepidermal layer (hour-glass cells), and *p*¹, *p*², parenchyma of spermoderm; *ep*, epidermis, and *p*³, parenchyma of endosperm; ×160.

usual in legumes, the outer cell walls are greatly thickened, showing a radiating cavity (*pal*¹) in surface view, while the inner portion of the cells has thinner walls and correspondingly broader lumen (*pal*²).

Subepidermal cells (*sub*).—These cells, although only about 6 μ high over the greater part of the seed, are very broad (upward of 30 μ) and are especially striking because of their prominent ribs clearly evident in surface view, where they present a beautiful radiating effect. In cross section they show evidence of the hour-glass form so characteristic of many legumes, the inner ends being broader than the outer.

The *parenchyma* (*p*) consists of several layers of compressed cells. The outer layers are of simple thin-walled parenchyma without intercellular spaces (p^1), while the inner layers are often distinctly spongy with evident intercellular spaces (p^2).

ENDOSPERM (fig. 5, *E;* fig. 6).—A simple epidermal layer (*ep*) containing aleurone grains is followed by several layers of large, more or less collapsed, empty cells with thin walls (p^3).

EMBRYO (fig. 5, *C*).—The cotyledons have a small-celled epidermis and mesophyll containing aleurone grains (*al*) but no starch. Palisade cells underlie the inner epidermis.

Red clover

Red clover (*Trifolium pratense* L.) is indigenous to Europe and is extensively grown for fodder in the United States, where it also grows spontaneously, having escaped from cultivation.

The pubescent stems are ascending, with 3-foliate toothed leaves, each oval leaflet often being notched at the apex and marked on the upper surface with a whitish spot. The rose-red flowers are borne in a dense sessile head closely surrounded by the uppermost leaves. The persistent calyx, with 5 bristle-like teeth and a bearded ring in the throat, is nearly as long as the delicate papilionaceous corolla, which is tubular below and withers after flowering. The pod differs from alfalfa in that it is one-seeded, straight, and flattened oval, with a very thin membranous lower half and hard caplike top. The seeds are slightly smaller than those of alfalfa, averaging 2 mm. in length. They are flattened kidney-shaped or rounded triangular with unequal sides one of which is concave. The color varies through light yellow to purple, the individual seeds being uniform in color or variegated.

HISTOLOGY

STEM.—The *epidermal cells* are longitudinally elongated with straight walls, often beaded especially just below the nodes, and a striated cuticle. Interspersed among these cells are numerous stomata and both unicellular and capitate hairs. The unicellular hairs, like those on the leaf, are long, thick-walled, warty, and

borne on a characteristic swelling of the epidermis having the appearance of an emergence.

Bast.—The only noteworthy tissues are the crystal-bearing cells accompanying the bundles of bast fibers and the large air spaces, below the unicellular hairs, such as occur on the leaf.

Phloem, *xylem*, and *pith* are similar to those of alfalfa.

LEAF.—The *upper epidermis* consists of approximately isodiametric cells with thin, gently wavy walls and scattered stomata. Hairs are absent.

Mesophyll.—The small bundles running through the simple parenchymatous ground tissue are accompanied by crystal-bearing cells, each cell containing a monoclinic crystal averaging 16 μ in length.

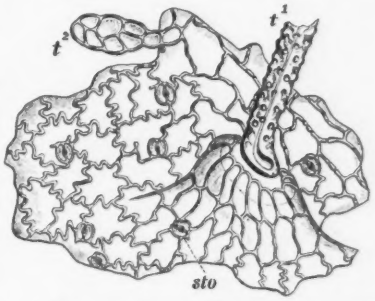

FIG. 7.—Clover (*Trifolium pratense*): lower epidermis of leaf with unicellular hair (l^1) arising from swelling of epidermis; l^2, capitate hair; *sto*, stoma; ×160.

The *lower epidermal cells* (fig. 7) have sinuous walls, the rather sharp bend of the waves being thickened and sometimes extended into the cell cavity as projections. Highly characteristic are the hornlike projections about the stomata (*sto*). The walls become slightly thicker and usually pitted toward the base of the leaf, especially over the veins. As on the stem, there are two forms of hairs, unicellular and capitate. The unicellular hairs (l^1) are stiff, very thick-walled and warty, varying in length up to 2 mm. and in diameter up to 30 μ. The warts are rather more prominent than on the corresponding hairs of alfalfa. They arise from a conical rosette of elongated cells over a large intercellular space, resembling in outward appearance an emergence. The capitate hairs (l^2), like those on alfalfa, consist of a stalk formed of a few cells in a single row and a club-shaped multicellular head.

CALYX.—The *outer epidermis* consists of simple cells with occasional wavy walls and numerous hairs both unicellular and capitate,

similar to those on the leaves and stem. The bristles, with papillae the whole length, end with a tuft of stiff unicellular hairs.

Mesophyll.—A single layer of crystal-bearing cells is conspicuous.

The *inner epidermis* is made up of wavy-walled cells and occasional hairs.

COROLLA.—The *epidermal cells* have very thin walls with papillae and striated cuticle toward the tip.

PERICARP.—The *epicarp* consists of sinuous-walled cells with scattered stomata. On the stem end the cell walls are thin, changing abruptly toward the tip to greatly thickened, sclerenchymatized and pitted walls.

The *mesocarp cells* are inconspicuous, with the exception of occasional scattered crystal-bearing cells.

SPERMODERM.—The *palisade cells* average 45 μ in height (running up to 55 μ over the radicle) and 7 μ in breadth. A narrow light line lies about 7 μ below the thin cuticle. They differ from the corresponding cells of alfalfa in that they are higher and the outer ends are flattened.

The *subepidermal cells* vary in height, but average 10 μ. They are upward of 20 μ broad and constricted in the center with lower ends broader than outer.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

Alsike clover

Alsike clover (*Trifolium hybridum* L.), although indigenous to Europe, has become very common in America. The plant branches, with erect stems bearing 3-foliate toothed leaves on long petioles and pedicellate flowers forming a loose round head on a long peduncle. Like alfalfa, the plant is described as smooth, though hairs are evident under a lens and are of diagnostic importance with higher magnifications. The membranous 5-cleft calyx is much shorter than the delicate rose-pink tubular corolla, which after flowering becomes brown and withering-persistent. The pod differs from that of alfalfa in that it is straight and from red clover in that it is 2–4-seeded. The greenish brown seeds are smaller

than those of alfalfa and red clover, reaching a length of 1.5 mm., but in shape resemble closely those of red clover. They are flattened rounded triangular with one concave side.

Histology

Stem.—The *epidermal cells* are thin-walled, pitted, and longitudinally elongated, with numerous stomata. The cuticle shows longitudinal striations. Occasional hairs both unicellular and capitate are present, the warts usually being indistinct.

Bast.—Conspicuous crystal cells are found in the bast just below the chlorophyll-bearing cells.

Leaf.—The *upper epidermis* consists of isodiametric cells, averaging 30 μ in diameter, with straight thin walls.

In the *mesophyll* the cells accompanying the bundles contain crystals averaging 15 μ in length.

Lower epidermis (fig. 8).—The cells are similar to those of the upper surface, the walls toward the leaf margins becoming gently wavy. Occasional unicellular (t^1) and capitate (t^2) hairs are present, the former being indistinctly warty and arising from a slightly thickened epidermal cell. They vary in length up to 800 μ.

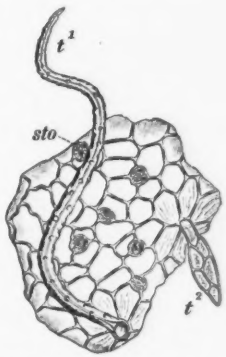

Fig. 8.—Alsike clover (*Trifolium hybridum*): lower epidermis of leaf with unicellular hair (t^1), capitate hair (t^2), and stoma (*sto*); ×160.

Calyx.—The *outer* and *inner epidermis*, with sinuous walls, bear capitate hairs similar to those on the leaf, also, at the base of the lobes and along their margins, unicellular thick-walled hairs with occasional indistinct warts.

Corolla.—The *epidermis* consists of elongated sinuous-walled cells with striated cuticle, papillae being present at the tip of the petals.

Pericarp.—The *epicarp* consists of transversely elongated slightly sinuous thin-walled cells, scattered stomata, and, especially at the margins, capitate hairs.

The *mesocarp* is but a few cells thick, except at the margins. Scattered crystal-bearing cells occur either singly or in groups.

The *endocarp* is made up of a single layer of thin-walled elongated cells.

SPERMODERM.—The *palisade cells* are 30–50 μ in height and 7 μ in diameter, with a narrow light line about 7 μ from the outer end. They differ from the palisade cells of alfalfa in that they are slightly higher, and from those of red clover in that they are rounded (not flattened) on the outer ends.

The *subepidermal cells* are not distinguishable from those of alfalfa and red clover.

The *parenchyma* consists of thin-walled collapsed cells.

The ENDOSPERM and EMBRYO are of simple structure of no diagnostic importance.

Identification in ground material

In a coarsely ground product, fragments of the leaves, flowers, pods, and seeds may be picked out and identified, but when powdered the unicellular hairs and crystals are the most conspicuous

	Alfalfa	Red clover	Alsike clover
Lower epidermis of leaf	Wavy walls	Deeply sinuous walls with projections at angles and about stomata	Straight walls
Unicellular hairs	Average diameter 15 μ, warts prominent	Average diameter 30 μ, warts prominent, arising from epidermal swelling	Average diameter 13 μ, warts indistinct

elements. Red clover may be distinguished from alfalfa and alsike clover by its larger, stiffer, and more numerous unicellular hairs arising from a swelling of the epidermis; alsike clover, from alfalfa and red clover, by the less distinct warts on the unicellular hairs.

The cell walls of the lower epidermis of the leaf are also characteristic, those of alsike clover being straight, of alfalfa simply

wavy, and of red clover very sinuous with projections at the angles and about the stomata.

A scheme for the identification of these three legumes by means of the epidermal cells of the leaf and the unicellular hairs is given in tabular form on the preceding page.

The palisade cells of the seed in alfalfa are not over 35 μ high, whereas in alsike and red clover they average somewhat higher. In red clover the outer ends of these cells are flattened, but in alfalfa and in alsike clover they are rounded.

CHICAGO, ILL.

THE RÔLE OF OXYGEN IN GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 181

CHARLES A. SHULL

A study of the respiration of *Xanthium* seeds was undertaken some time ago with the purpose of determining whether there was any change in the permeability of the seed coats to oxygen during the period following the normal ripening of the seeds. Some evidence was noted previously[1] that there was either a change in permeability of the seed coat, or a change in oxygen need of the embryo during the early winter, and it was believed that a careful measurement of the oxygen used by the seeds with coats on and off at successive intervals during the year would show which of these changes occurred, and at what period of the ripening process. Circumstances have prevented the carrying out of this series of tests; but the preliminary results are of sufficient interest in connection with the rôle of oxygen in germination behavior to warrant placing the data on record. The measurements were made with a respirometer of excellent type designed by Dr. WILLIAM CROCKER, to whom I am further indebted for suggestions regarding the problem. The respirometer was kept in a Freas thermostat at 25.25° C., and the volumes of oxygen used are reduced to standard conditions. Seeds of *X. glabratum* in dry storage for nine months were used.

First it was necessary to know what part of the oxygen was used by the coats under ordinary atmospheric germinative conditions. Two lower seeds were placed in one chamber of the respirometer, and the coats of two lowers in the other chamber. In 22.5 hours the two seeds used 0.475 cc. of oxygen, while the two coats used 0.098 cc. From the results of BECQUEREL'S work[2] I had suggested that the coats were probably responsible for a part

[1] SHULL, CHAS. A., The oxygen minimum and the germination of *Xanthium* seeds. BOT. GAZ. 52:453–477. 1911.

[2] BECQUEREL, PAUL, Recherches sur la vie latente des graines. Ann. Sci. Nat. Bot. IX. 5:193–320. 1907.

of the respiration of intact seeds. That part is now shown to be considerable, amounting in this instance to 20 per cent of the total.

The respiration of lower and upper seeds with coats intact under atmospheric conditions of germination was compared, with the result that two lowers used 0.687 cc. and the two uppers 0.509 cc. of oxygen in 42.3 hours, a ratio of lowers to uppers of 1.35:1. It should be said that upper seeds always weigh less on the average than the lowers; and in using equal numbers of seeds the weight of respiring substance is somewhat less in the uppers.

The respiration of the lowers with coats on and coats off in ordinary atmosphere is especially interesting (see table I).

TABLE I

Time	Oxygen used		Ratio
	Coats off	Coats on	
10 hours.....	0.500 cc.	0.3615 cc.	1.38:1
10–15 "	0.478 "	0.1025 "	4.65:1
15–17.25 "	0.316 "	0.040 "	7.9 :1
Total.....	1.294 "	0.504 "	2.57 :1

TABLE II

Time	Oxygen used		Ratio
	Coats off	Coats on	
5 hours..... ...	0.1609 cc.	0.075 cc.	2.14:1
5–15 "	0.618 "	0.1107 "	5.58:1
Total, 17.25 hours...	0.9825 "	0.1947 "	5.046:1

A similar test with uppers is shown in table II. The coats were not placed in the chambers with the embryos where coats were off, so that the ratios in the tables are too low as regards actual embryo respiration. The rapid increase of use of oxygen by naked embryos as germination commences is well illustrated.

Finally, the oxygen used by uppers and lowers with coats on, in an atmosphere 96 per cent oxygen, was compared, with significant results. Two lower seeds used 1.007 cc. in 12.5 hours, while the

uppers used but 0.4406 cc., a ratio of 2.28:1. A repetition of this experiment resulted in the lowers using 1.257 cc. in 22 hours, the uppers 0.772 cc., a ratio of 1.63:1. Invisible defects in coats might cause some variation in these ratios, but they are believed to approximate average results.

It is interesting to note that two lowers in atmosphere used 0.687 cc. of oxygen in 42.3 hours, while the same number used 1.007 cc. in 12.5 hours in 96 per cent oxygen; and that uppers which used 0.509 cc. in 42.3 hours used 0.4406 cc. in 12.5 hours in 96 per cent oxygen. The increased oxygen pressure causes a large increase in the oxygen intake of both seeds with coats intact, but exerts the greater influence on the lowers. The relation between oxygen influence and respiration seems to be close. At least we may say that the conditions of the oxygen supply which lead to increased use of oxygen are just the conditions which bring about germination. The possibility that oxygen exerts its stimulative effect on germination by increasing respiration, thereby yielding more energy, is strongly suggested, without, however, precluding the possibility that other effects correlated with increased respiration might determine its influence in germination.

BECKER[3] recently tested the influence of oxygen on the germination of seeds of several plants. The fruits of *Dimorphotheca pluvialis* were found to germinate more readily in O_2 than in air, the ray seeds especially showing the favorable influence. Short exposures to oxygen (15 hours) had no such effect, but if the time of exposure were lengthened to 30 hours, this exposure favored further germination under atmospheric conditions. The ray seeds again showed the effect more strongly than the disk seeds. When the fruit and seed coats were removed, 10 hours' exposure to oxygen affected germination favorably, but 13 hours' lengthened the germination time. The seeds of *Calendula eriocarpa* with coats intact were greatly favored by oxygen, while the yellow-brown vertical fruits of *Atriplex hortensis* and *A. nitens* showed an injurious effect from increased oxygen pressures. The relation of oxygen to germination in these cases seems to be irregular and inconstant.

[3] BECKER, H., Über die Keimung verschiedenartiger Früchte und Samen bei derselben Species. Beih. Bot. Centralbl. **29**[1]:21–143. 1912.

BECKER draws a general conclusion from his results that oxygen acts as a stimulus, and takes particular exception to the idea advanced by CROCKER[4] that the oxygen increases the respiration and in this way initiates germination. In reviewing BECKER's paper,[5] I stated that there was no doubt that in *Xanthium* the oxygen was actually used in germination, and suggested that increased respiration might be identical with BECKER's stimulus. While it is entirely possible that the oxygen influence is exerted through some other process correlated with increased respiration, the data presented here give the ground upon which that suggestion was based. Unfortunately, BECKER's work gives us no data as to the respiration behavior of the seeds on which he worked, so that no comparison with the behavior of *Xanthium* seeds can be made at present.

LEHMANN,[6] in discussing the possibility that O_2 might act as a catalyst, accepts BECKER's idea that oxygen acts as a chemical stimulus, not merely by increasing respiration. Of course, the word "stimulus" is vague and indefinite. But it should not imply an additional absorption of oxygen, for this could not occur without involving oxidation of some kind, which would be respiration. Even if oxygen is conceived to be a catalyser, that conception does not involve increased use of O_2, for catalysts are not used in the processes they carry on.

The biological rôle of oxygen is so complex that we may not say its effect is always due to increasing respiration or oxidation. The rôle, indeed, may be different in different seeds and plants. For instance, ÁRPÁD PÁÁL[7] claims that reduction of oxygen pressure even to 0.75 normal lengthens geo-presentation and geo-reaction time to a marked degree. This work of PÁÁL's still awaits confirmation. And although the earlier work of STICH, JOHANNSEN, and others indicates that this amount of reduction

[4] CROCKER, WM., The rôle of seed coats in delayed germination. BOT. GAZ. 42:265–291. 1906.

[5] BOT. GAZ. 54:433. 1912.

[6] LEHMANN, E., und OTTENWÄLDER, A., Über katalytische Wirkung des Lichtes bei der Keimung lichtempfindlicher Samen. Zeitschr. Bot. 5:337–364. 1913.

[7] PÁÁL, ÁRPÁD, Analyse des geotropischen Reizvorgangs mittels Luftverdünnung. Jahrb. Wiss. Bot. 50:1–20. 1911.

should not affect the rate and nature of respiration, yet it would be very desirable to repeat PAÁL's work, studying the rate of respiration along with presentation time and reaction time to discover whether there is a parallel effect with that on the respiration rate and germination power of seeds.

ZALESKI[8] has noted the influence of oxygen on the rate of protein synthesis. IVANOFF[9] has shown that oxygen is necessary for the transformation of zymogen into zymase, and, as is well known, there are a number of oxygen carriers and oxygen absorbers in the living cell. Xanthophyll and other pigments absorb oxygen, lecithin plays a similar rôle, and PALLADIN[10] has now shown that his respiratory chromogens take up oxygen readily. All of these facts go to show how complex the oxygen rôle may be, and suggest some of the possibilities of even brief exposure of seeds to oxygen.

On the other hand, however, it would be strange if the oxygen effect in some cases were not due simply to its influence upon respiration. The influence of the amount of oxygen present on aerobic and anaerobic respiration, which differ so markedly in the amount of energy released, is well known. Anaerobic may change over to aerobic on access of oxygen, with a consequent rapid rise in energy releasal leading to germination.

With these *Xanthium* seeds it has been shown that when the oxygen supply is increased, it in some way brings about an immediate and rapid increase in the rate of oxygen absorption. At the same time, the increased oxygen supply brings about an immediate germination of the seeds. The two effects, increased absorption and germination, are closely correlated as regards their relation to the oxygen supply. This shows conclusively, I believe, that the assumption made by BECKER and LEHMANN, which led them to reject the idea that the influence was exerted through increased respiration, is not correct, so far as *Xanthium* is concerned. Nor does their work throw any light on this particular point, since they

[8] ZALESKI, W., Zur kenntnis der Stoffwechselprocesse in reifenden Samen. Beih. Bot. Centralbl. **27**:63–82. 1911.

[9] IVANOFF, L., Über die sogenannte Atmung der zerriebenen Samen. Ber. Deutsch. Bot. Gesells. **29**:563–570. 1911.

[10] PALLADIN, W., and TOLSTAJA, Z., Über die Sauerstoffabsorption durch die Atmungschromogene der Pflanzen. Biochem. Zeitschr. **51**:381–397. 1913.

did not measure the respiration of the seeds on which they worked. However, owing to the complexity of the oxygen rôle in physiological processes, it is very difficult to say just which function or functions are affected. It seems certain that the oxygen acts as a limiting factor on some function, whether by limiting the process of respiration or energy releasal, by limiting enzyme formation or the action of oxygen carriers, or in other still less definite ways. The exact method by which absence of oxygen delays germination can be determined only by further investigation. In the meantime theories may well await the facts which will make philosophical discussion of this question unnecessary.

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BRIEFER ARTICLES

A METHOD OF HANDLING MATERIAL TO BE IMBEDDED IN PARAFFINE

(WITH ONE FIGURE)

The usual method of handling material to be imbedded in paraffine is slow and tedious, and attended with some danger of damage to the material. Ordinarily, each batch is killed in a separate bottle, in which it remains up to and even through the paraffine bath. In washing, a cloth is usually tied over the end of the bottle, which is put under a running tap or thrown into a vessel of running water. Either way results in washing of questionable thoroughness. Then the various grades of alcohols and xylols are pipetted on and off, a very tedious process with small light objects that are likely to be drawn into the pipette and injured or lost.

After trying several devices, the writer has had success in eliminating these difficulties by the following method: About 6.25 cm. lengths are cut from ordinary medium thickness glass tubing of the desired diameter. One end is heated and slightly flared. Over the flared end is tightly tied a piece of thin cloth, and the excess of cloth and string closely trimmed away (fig. 1, *A*). With a stout cord, a slip knot (*B*) is quickly tied and drawn up, and holds the cloth firmly. The material is placed in this cloth-bottom tube, and remains in it through all the processes up to the paraffine bath. If the objects are large or numerous, it is better to kill in an ordinary bottle and transfer to the tube just before washing.

In washing, the tube of material is placed in a vessel of less height (such as a small salt mouth bottle), and water siphoned into the tube from a supply vessel directly under the running tap (*C*). The tube end of the siphon is drawn out fine, allowing only a small amount of water to pass over. A section of rubber tubing in the siphon gives greater flexibility. Because the water level is high in the tube, and all the fresh water flows over the material, washing is very thorough. Under all conditions the stream of water is uniform and gentle, even with considerable variation in tap pressure, as we have in Allahabad. Any number of tubes, each with a separate siphon, may be grouped around the supply vessel and washed at one time.

The series of alcohols and xylols is prepared in tightly stoppered bottles and arranged in sequence. The tubes of washed material are placed in the bottle of lowest grade alcohol and transferred at proper intervals from bottle to bottle through the series. Any number of tubes are easily and rapidly handled by means of a pair of forceps. There is no danger of losing small objects, because the top of the tube is always

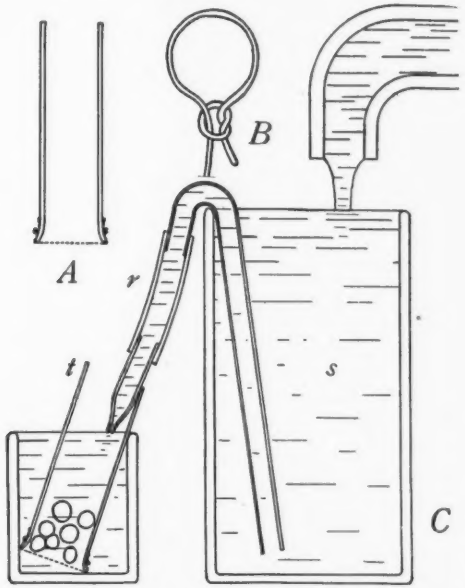


FIG. 1.—Sectional diagrams: *A*, end of tube flared and covered with cloth; *B*, rapid method of tying cord; *C*, arrangement for washing material; water flows from the tap into the supply vessel (*s*), through the siphon into the tube of material (*t*), through the cloth bottom, and overflows from the small vessel; a piece of rubber tubing (*r*) in the siphon adds flexibility.

above the level of the reagent. With proper labels on the series bottles, the exact location of the material, and the next change, are always known at a glance. The material is left in the tubes through all the processes up to the first bath in melted paraffine.

The advantages of this method are as follows: there is no loss of time in handling large quantities of small light objects; washing is easy and thorough; there is no danger of injury to the most delicate material; no

labels, except those for identification, are necessary on the separate batches of material; and the series of alcohols and xylols may be used repeatedly, for while there is a continual weakening of each grade in the series, the weakening is proportional throughout the whole series, so that their relation to each other remains practically unchanged.—WINFIELD DUDGEON, *Ewing Christian College, Allahabad, India.*

THE RELATION BETWEEN THE TRANSPIRATION STREAM AND THE ABSORPTION OF SALTS

During the winter of 1908–1909 experiments were conducted at Santiago de las Vegas, Cuba, in order to determine the comparative transpiration of tobacco plants under cheesecloth shade and in the open ground. For this purpose plants were grown in galvanized iron tanks which were set into outer incasing tanks permanently sunk in the ground. Six tanks were placed among the plants of a field of tobacco grown under cheesecloth, and six were set in an adjoining tobacco field not shaded. The quantity of water transpired by the plants in the tanks was determined by daily weighings, the quantity transpired being replaced each day. At maturity the leaves, stems, and roots of each plant were harvested separately, dried, and ground. The ash was determined in water-free samples of the ground material. From the data the total ash of the plants was calculated. The condensed results of the experiment are given in the following table.

PLANTS GROWN IN THE OPEN

No. of plant	Total water-free substance produced, in grams	Total water absorbed, in cc.	Total ash in plants, in grams	Water absorbed per gram of ash
1	209.03	52,066	18.19	2862
3	168.33	42,059	16.74	2512
5	191.91	46,840	20.54	2280
6	187.97	45,418	16.74	2713
7	185.06	46,447	18.86	2463
9	188.20	45,234	18.42	2456
Average	188.42	46,344	18.25	2548

PLANTS GROWN UNDER SHADE

No. of plant	Total water-free substance produced, in grams	Total water absorbed, in cc.	Total ash in plants, in grams	Water absorbed per gram of ash
10	211.43	42,122	21.36	1972
12	199.08	38,256	21.88	1748
14	184.67	36,448	20.15	1809
15	172.56	33,965	19.91	1706
16	186.80	33,922	21.56	1573
18	174.27	32,407	21.61	1500
Average	188.14	36,187	21.08	1718

From this table it appears that the total dry substance produced by the plants was about equal in the two sets.

The plants grown in the open absorbed about 28 per cent more water than those grown under shade. The plants which absorbed and transpired the greater quantity of water contained both the smaller percentage and the smaller absolute quantity of ash.

It appears, therefore, that the absorption of salts by roots is independent of the absorption of water, and that the transpiration stream does not exert an accelerating effect on the entrance of salts.—HEINRICH HASSELBRING, *Bureau of Plant Industry, Washington, D.C.*

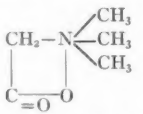
CURRENT LITERATURE

BOOK REVIEWS

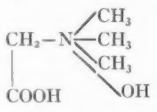
The simple plant bases

A recent book by TRIER[1] will interest students of plant physiology and plant chemistry.

The plant bases are divided into three classes: (1) the high molecular, physiologically active alkaloids peculiar to certain plants; (2) the simple alkaloids without known peculiarities and widely distributed in the plant kingdom; (3) the basic splitting products of the protoplasmic constituents, as proteins, nucleic acids, lecithins, etc. Plant alkaloids are nitrogen-containing bodies which arise in the formation or transformation of protoplasmic substances. By synthetic processes the reaction capacity of their basic H atom is locked up in such a way as to render them unavailable for resynthesis of protoplasmic substances. The primary amines are the simplest alkaloids. They are formed by the breaking up of the carboxyl group of the corresponding amino acids. The simplest case would be the removal of a molecule of CO_2. The amines of nearly all of the amino acids are known. They are seldom found in higher plants as they are at once converted into higher alkaloids either by condensation or by the simple process of methylation, which is a very general process in plants and has the effect of throwing the methylated body out of the field of chemical activity. The betains are completely methylated amines. This relates them directly to the amino acids. They are simple alkaloids and may be further defined as substances in which one assumes an intra-molecular saturation of the amino group with the acid carboxyl group. They cannot replace cholin in the lecithin molecule.



Betain (glycocoll betain)



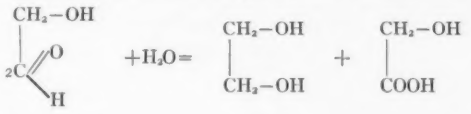
(hydrated form)

Cholin is formed by a methylation process of colamin (aminoethylalcohol), which is the primary amine of serin. This methylation occurs within the lecithin complex; therefore it is not a primary building stone of lecithin but appears only as a hydrolytic product of this substance. Xanthin bases also undergo methylation, as caffein from xanthin.

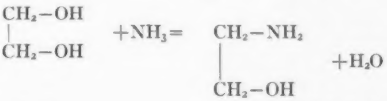
The hypothesis formulated to explain the formation of the simplest amino

[1] TRIER, GEORG, Über einfache Pflanzenbasen und ihre Beziehungen zum Aufbau der Eiweisstoffe und Lecithine. pp. iv+117. Berlin: Gebrüder Borntraeger, 1912.

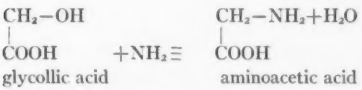
acid includes also an explanation of the primary origin of characteristic building stones of lecithins, since the acids of the proteins and the alcohols of the lecithins arise through one and the same reaction. This would explain STOKLASA'S finding that protein and lecithin-formation always run parallel. The formaldehyde, formed by the reduction of CO_2 in green plants, is condensed to glycolaldehyde. By the Cannizzaro reaction, one molecule of glycol and one molecule of glycollic acid arise from two molecules of glycolaldehyde. These products furnish aminoethylalcohol and glycocoll by aminization.



glycolaldehyde glycol glycollic acid



glycol aminoethylalcohol



glycollic acid aminoacetic acid (glycocoll)

This reaction furnishes, therefore, the simplest amino acid, the mother substance of the simplest betain and aminoethylalcohol which may give rise to cholin by methylation as noted above. Analogous with the above reaction a furthur condensation of formaldehyde is postulated for the formation of glycerinaldehyde, and from this may arise glycerin and serin by the reaction of Cannizzaro and by aminization. We now have the glycerin for the formation of fats, lecithin, and other phosphatids. The higher amino acids may be considered derivatives of serin, and alanin a reduction product of the same. The author denies the probability of HCN being an intermediate substance in the primary formation of proteins.

The author extends his hypothesis to the mechanism of methylation. By the reaction of Cannizzaro, formaldehyde can furnish methyl alcohol and formic acid. The methyl alcohol can in turn furnish the alkyl groups for the methylation of metabolic products. Since this scheme is based upon the work of the chlorophyll apparatus, it will not explain the mechanism of the methylations, which frequently occur in animals.

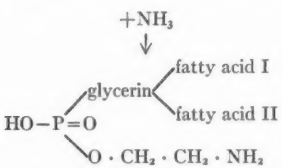
The Cannizzaro reaction is accelerated by the enzyme aldehydase, which has been definitely proven in this case to be a hydrolytic enzyme. In addition to this, if oxygen is present, the alcohols formed at the same time can be oxidized. We have here a case of an enzyme accelerating both hydrolysis and oxidation.

Asparagin, the amide of aspartic acid, has usually been given an important place in studies of protein metabolism in plants. SCHULZE has shown that it must be formed at the cost of amino acids, and according to his investigations it is a secondary product of protein changes and is not a primary building stone of proteins. TRIER cites considerable evidence in support of his contention that aspartic acid, the mother substance of asparagin, is formed from glutamic acid and leucine by oxidation processes. This agrees with the fact that where there is a strong accumulation of asparagin in germination the seed proteins show a high content of glutamic acid.

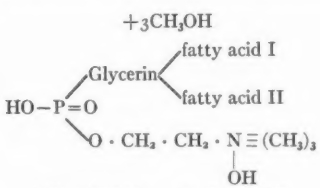
The author's views regarding the part played by the carbohydrates in phosphatid preparations are of great interest, since it is impossible at the present time to fit them into any scheme of lecithin constitution, although TRIER himself has proved that phosphatids from seeds contain reducing substances in chemical combination. He also found that the fatty acids increase and the phosphorus decreases as the content of reducing substances increases. This led the author to think that the regular phosphatid of STRECKER and HOPPE-SEYLER must be accompanied in plants by a substance of the character of animal cerebrosides, that is, substances which furnish, as hydrolytic products, fatty acids and nitrogen bases but no phosphoric acid. The following scheme shows the gradual building up of lecithins according to TRIER:

Formaldehyde → Glycolaldehyde → Glycol → Diglyceridphosphoric acid glycol ester (N free lecithin)

Triglyceride → Diglyceride → Diglyceridphosphoric acid → Diglyceridphosphoric acid glycol ester (N free lecithin)



Diglyceridphosphoric acid-aminoethylester (cholamin lecithin)



Ideal lecithin or cholin lecithin

Those interested in plant physiology and plant chemistry will find this little book of great value on account of the many positive facts stated and because of the critical way in which the author has attempted to organize these facts. Some of the hypotheses may be more sweeping than the facts warrant, but they should serve to stimulate work on these important but difficult problems.—CHAS. O. APPLEMAN.

MINOR NOTICES

Michigan trees.—The first thing that recommends this little manual[2] to the student of trees is its convenient pocket size (5 by 7.5 inches), which makes it more readily useful in the field than more pretentious volumes. A closer examination reveals the fact that it is well illustrated by carefully made drawings of the leaves, flowers, buds, and fruit of each species. The keys seem to have been constructed with more than usual care, and are in duplicate, one based largely upon the leaves, for use during summer; and a second making use of the bud and twig characters as a basis of identification during the winter. In order that the bulletin may appeal to as large an assemblage of readers as possible, the use of technical terms has been reduced to a minimum, and those necessarily employed are fully explained in a glossary. The arrangement of drawings and descriptions of species upon pages facing one another adds to the ease with which the manual may be consulted.—GEO. D. FULLER.

NOTES FOR STUDENTS

Mutations and inheritance in Oenothera.—DAVIS[3] has recently reported a continuation of his studies of *Oenothera*. In previous papers[4] he has described the F_1 and F_2 generations of hybrids between *O. biennis* and *O. grandiflora*. The present account deals with the behavior of F_2 and F_3 generations of the same or similar hybrids. The data presented are discussed (1) from the standpoint of their bearing upon the origin and habit of mutation of *O. Lamarckiana*, and (2) with relation to their possible interpretation by Mendelian principles of inheritance. The latter, if one may judge from the methods employed in these studies, has been incidental to the former. The primary purpose of the investigations has been to determine the possibility of the synthesis through hybridization of a type similar in both taxonomic features and mutating habit

[2] OTIS, CHARLES H., and BURNS, G. P., Michigan trees. 12mo. pp. xxxii+246. *figs. 120*. Ann Arbor: Univ. Mich. Bull. N.S. **14**: no. 16. 1913.

[3] DAVIS, B. M., The behavior of hybrids between *Oenothera biennis* and *O. grandiflora* in the second and third generations. Amer. Nat. **47**:449–476, 547–571. 1913.

[4] ———, Notes on the behavior of certain hybrids of *Oenothera* in the first generation. Amer. Nat. **44**:108–115. 1910; Some hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. **45**:193–233. 1911; Further hybrids of *Oenothera biennis* and *O. grandiflora* that resemble *O. Lamarckiana*. Amer. Nat. **46**:377–427. 1912.

to *O. Lamarckiana*. The results are summarized briefly as follows: "I have not been able to synthesize by direct crosses, from wild stock so far obtained, any hybrid with all of the characters of *Lamarckiana* in the same plant, although I believe that all of the important taxonomic characters of *Lamarckiana* have been represented in some of my hybrids. . . . . The resemblance of my various hybrids to *Lamarckiana* and the parallelism of their behavior in the F_2 and F_3 to that of *Lamarckiana* give in themselves sufficient reasons, in my opinion, to justify the belief in its hybrid character and to point to the probability that this plant arose as a cross between distinct forms of *Oenothera*. *Lamarckiana* thus would not be representative of a wild species of essentially stable germinal constitution and its mutations are most simply interpreted as the behavior of a hybrid." Prominent among the types that appeared in F_2 and again in F_3 and bred true in a later generation—this behavior constituting the similarity to the mutation habit of *O. Lamarckiana*—were dwarf forms with narrow etiolated foliage, and somewhat similar dwarf forms with normal green foliage. These forms occurred in approximately 10 per cent and 15 per cent of their respective families. Other striking forms were a *semigigas* type with at least 21 chromosomes, and a form similar to *O. elliptica*.

With relation to a possible Mendelian interpretation of his results, DAVIS' most troublesome problems are: (1) "the explanation of the large groups of dwarfs thrown off in the F_2 generations and repeated by certain plants in the F_2," and (2) "the explanation of the well-defined progressive evolution, excluding the dwarfs, exhibited by these same cultures." This "progressive evolution" consisted in the appearance of F_2 families whose flower size ranged from about 1 cm. greater than that of the large-flowered parent (*grandiflora*), to about twice as large as that of the small-flowered parent (*biennis*), and in a corresponding increase in size and amount of crinkling of the leaves. DAVIS doubts the possibility of explaining this advance in flower size on the basis of a recombination of size factors as suggested by the multiple-factor hypothesis, because there was in these families no balancing group with flowers smaller than the small-flowered parent, and he inquires: "What had become in these cultures of the factors responsible for small size?" When it is noted, however, that at least one F_2 family exhibited pronounced "retrogressive evolution" in flower size, little difficulty will be experienced by students of the inheritance of quantitative characters in interpreting DAVIS' results by means of the multiple-factor hypothesis. The obvious suggestion is that the several F_1 plants tested had somewhat different combinations of size factors, that is, that one or both of the parents were heterozygous with respect to a few quantitative factors, a condition which could scarcely be detected under the masking effect of ordinary fluctuation without resort to a careful quantitative study of several lines of progenies of the plants used as parents of any hybrid.

The dwarf plants in certain F_2 and F_3 cultures are also inexplicable to DAVIS on the basis of a recombination of size factors, and with this the reviewer is inclined to agree. The dwarfs are much smaller than the parents, the gap

between them and the parents or the smallest of other F_2 forms is not bridged, and there are no compensating giant forms. It seems more likely that these plants are dwarfs because of some abnormality of function, and that, so far as size factors are concerned, they may be potentially tall. One group in fact is characteristically etiolated. The abnormalities, however, may well be due to a recombination of other genetic factors present in the parents. The fact that the dwarfs have occurred in some cultures in ratios much above 1:15 need occasion little worry at the present stage of the investigations, since students of genetics are coming to look to ratios merely for indications, and to base conclusions rather upon a factorial analysis of the material, worked out by intercrosses of the diverse types of the culture concerned or by back crosses with the parents.

DAVIS' results as a whole are of the greatest importance. It is hoped that he will find the time in the near future to subject his material to statistical and factorial analysis in the same painstaking way that has given the brilliant results secured in his attempted synthesis of *Oenothera Lamarckiana*.

HERIBERT-NILSSON[5] has reported the results of a study of *Oenothera Lamarckiana* and its derivatives. His cultures exhibited numerous heritable differences with regard to such characters as color of leaf veins and leaf blades, breadth of flowers, length of fruits, and height of plants. The appearance of these minor forms in cultures of *Lamarckiana* is regarded as an indication that the species is not a constant one. The characteristics by which these forms are distinguished are the same, in part at least, as those that serve to differentiate the mutations of *Lamarckiana*. This fact suggests to the author that the mutations are the result of new combinations of characters, or factors, present in the parent species.

The mutations that appeared in HERIBERT-NILSSON's cultures were not identical with those of DE VRIES. Some of them were entirely unlike DE VRIES' mutations, while others were parallel types. Some of the latter resembled *rubrinervis*, *gigas*, *albida*, and *lata*, for instance, in certain respects, but differed from them in others. Certain of the author's mutants combined important characteristics of several of DE VRIES' mutants. One, for instance, exhibited characters of *rubrinervis*, *scintillans*, and *lata*. The forms studied differed principally in quantitative characters, a fact that makes a factorial analysis a matter of extreme difficulty. The behavior of certain qualitative characters, particularly red color of leaf veins, indicated that two or three Mendelian factors might be concerned. Ratios of red-veined to white-veined individuals occurring in the F_3 generation of a giant form approached 3:1, 15:1, and 63:1. A complete factorial analysis of these groups, based upon a study of their progenies, has apparently not as yet been attempted. HERIBERT-NILSSON's suggestion that giant (*gigas*-like) oenotheras have arisen through

[5] HERIBERT-NILSSON, H., Die Variabilität der *Oenothera Lamarckiana* und das Problem der mutation. Zeitsch. Ind. Abst. Vererbungs. **8**:89–231. 1912.

the combination of numerous independent size factors is criticized by GATES[6] (1) on the basis of cytological evidence (tetraploid chromosomes) to the contrary, and (2) on the basis of their sudden, discontinuous origin.—R. A. EMERSON.

Araucarians.—Miss HOLDEN[7] has recently described the stems of two fossil plants from eastern Canada, a *Tylodendron* from the south shore of Prince Edward Island and a form which she claims is *Voltzia coburgensis* from the Triassic at Martin's Head, New Brunswick. She has identified her specimens by the casts of the pith, and by the structure as well, and uses her determinations as evidence of the geological horizon of the strata in which they are found. In this connection, she states: "Since *Tylodendron* is characteristic of the Permian, there can be no question that these strata [those of Prince Edward Island] are of that age"; and of *Voltzia:* "Paleobotanical evidence indicates that the Mesozoic strata of New Brunswick are of the same age as those of the eastern United States, and should be correlated with the Lettenkohle or Lower Keuper of Europe." The pith casts of her *Tylodendron* are typical, and she states of the ligneous structure: "It agrees exactly with that described by DAWSON from Mr. BAIN's specimen as *Tylodendron Baini*, and with that described by POTONIÉ as *Araucarioxylon rhodeanum* Goepp."

In discussing the evidence for and against the generally accepted view of the araucarian affinity of *Tylodendron*, a view, however, from which Miss HOLDEN dissents, she agrees with POTONIÉ that "the nodal swellings and instanding protoxylem strands causing the ridges and furrows of the pith casts are identical with similar structures in *Araucaria* and *Agathis*," but states that instanding protoxylem strands are common to all living conifers. She admits that the medullary rays are typically araucarian, the rays uniseriate, rarely over 10 cells high, and composed of thin-walled cells, but she says that all conifers have uniseriate rays. Neither of her arguments, however, precludes the araucarian connection. Of the tracheary pitting, she says: "Its closely compressed and alternating pits clearly affiliate it with *Araucarioxylon* Krauss," but she considers that this does not indicate araucarian affinity, since "closely compressed and alternating pitting is not the primitive condition for the Araucarineae." This statement is made on the authority of Professor JEFFREY's[8] recent work. While both of these articles were in press, however, the writer[9] advanced evidence

[6] GATES, R. R., Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. **33**:92–99, 113–150. 1913.

[7] HOLDEN, Miss R., Some fossil plants from eastern Canada. Ann. Botany **27**: 243–255. 1913.

[8] JEFFREY, E. C., The history, comparative anatomy, and evolution of the *Araucarioxylon* type. Proc. Amer. Acad. **48**:531–571. 1912.

[9] THOMSON, R. B., On the comparative anatomy and affinities of the Araucarineae. Phil. Trans. Roy. Soc. B **204**:1–50. 1913.

to show that the reverse is true. The pitting, for example, in the *cone axis* of *Araucaria Bidwillii* may be as much as 5-seriate, the pits alternating and extending from end to end of the tracheid. In this and in other primitive regions as well, the mouth of the pit is elliptical, a vestige of the more ancient scalariform condition, a condition which is retained even longer where the medullary ray touches the tracheid. No torus is present in these regions too, although this is well developed in the whole pine alliance. Again, Miss HOLDEN says: "Impressions present more evidence for merging *Tylodendron* with the araucarians. Several varieties of leafy branches, known as *Walchia*, and definitely associated with *Tylodendron* pith casts, have been described, all bearing a close resemblance to different species of *Araucaria*. Of their fructifications little is known, further than that, as shown by ZEILLER, the scales of the female cone bear single seeds, another araucarian feature." She presents nothing in opposition to the above statement, but in concluding the paragraph says: "If these criteria are reliable, the presence of *Tylodendron* in the Permian strata bears out the orthodox view that the Araucarineae are the oldest living family of the Coniferales." Since Miss HOLDEN has not invalidated any of these criteria, the case must hold for the araucarian connection. She evidently fears to draw this conclusion on account of the temerity of the advocates of araucarian ancestry of the conifers, for her final point is that "there are woods of the *Tylodendron* type extending as far back as the Culm, yet no advocate of the antiquity of the araucarian line would suggest that it extends as far back as that."

On the other hand, Miss HOLDEN considers that her more recent form *Voltzia coburgensis* from the Triassic is an araucarian, but one derived from the Abietineae. She accepts the conclusion as to the araucarian affinity of *Voltzia*, though she has rejected this conclusion in the case of *Tylodendron* which has one point more in its favor. The character of the rays, etc., of *Voltzia* is described as distinctly araucarian, just as in *Tylodendron*. The leaf trace is single at the pith, but forks during its passage through the wood, a similar condition, as Miss HOLDEN states, to that in *Agathis*. She has previously (p. 246) drawn attention to "the araucarian single trace" in *Tylodendron*. Of the pits in *Voltzia*, she says that they are "always uniseriate and usually scattered . . . . rarely are they so closely compressed as to be flattened and angular. While they are as distant as the pits of the Abietineae and Taxodineae, they are never, as is the rule in these groups, separated by so-called bars of Sanio." This pitting is the one point of difference from *Tylodendron*, where the pits are typically of the *Araucarioxylon* type, and the one point more in favor of the araucarian connection if the latter.

The anatomical evidence of abietinean affinity of *Voltzia* is said to be "the scattered position of the pits." Why this is distinctive of the Abietineae is not clear. She herself states (see the quotation in the preceding paragraph) that it is found in the Taxodineae, and it occurs in other conifers as well. Nor is it evident why the cone is abietineous, as Miss HOLDEN states. She refers to nine authorities, only one of which agrees that it is abietineous. Three refer

it to the Cupressineae and four put it with the Taxodineae, as the original describer, BRONGNIART, also did. No reasons are given for Miss HOLDEN'S choice.

After discussing the combination of araucarian and abietinean characteristics in *Voltzia*, she speaks of other forms showing similar combinations, and says: "Dr. JEFFREY . . . . appears to have demonstrated that the Abietineae are older, and that it is the Araucarineae which become progressively more and more like the Abietineae in successively older geological formations." Certainly this is not the case in the two forms she describes, even disregarding the evidence from the cone in both cases which is known in impression only. When, however, the cone impressions are given equal importance in each case, the foregoing conclusion is further at variance with the facts. Nor is the case improved by including the other transitional forms, which are considered important by the Harvard school, *Woodworthia* of the Triassic and *Araucariopitys* of the Cretaceous, since the former is practically an araucarian and the latter an abietinean. So far then as the evidence from the transitional forms stands, the reverse of the conclusion attributed to Professor JEFFREY is the fact. It is the Abietineae which are more like the Araucarineae in the older geological formations. When this evidence is taken in connection with the fact that no true Abietineae have been described from the strata preceding the Triassic, the historical evidence is seen to be wholly adverse to the Abietineae.

Miss HOLDEN'S own work then, far from supporting the abietinean ancestry of the Araucarineae, is directly opposed to it. Had the full evidence of the character of the ancestral pitting in the araucarians been before her, she would probably have escaped the pervasive influence of this theory.—R. B. THOMSON.

Pityoxylon.—One of Miss HOLDEN'S[10] three new species of Pityoxyla from the Middle Cretaceous of Cliffwood, N.J., is "probably the earliest form with all the characters of a modern hard pine, yet retaining certain ancestral features, as the association of primary and fascicular leaves." She has appropriately designated this form *Pinus protoscleropitys*. Its occurrence in the Middle Cretaceous is regarded as "an argument for the great geological antiquity of the pines as such." Her *Pityoxylon foliosum* is "possibly the wood of *Prepinus*, with all its leaves borne directly on the main axis," and combining the characteristics of both hard and soft pines. The third form, *Pityoxylon anomalum*, has much the same type of wood structure as the second, but has "all its leaves borne on short shoots."

The spur shoots are described as large in both forms, "much larger than those of living pines," but unbranched, as in modern pines, and thus unlike those of *Gingko*, or *Woodworthia* from the Triassic whose spurs were also large. The large size of the spurs in the old fossil forms is evidence that the spur was ancestrally a branch.

[10] HOLDEN, Miss R., Cretaceous Pityoxyla from Cliffwood, New Jersey. Proc. Amer. Acad. **48**:609–623. 1913.

The resin canals, both horizontal and vertical, are said to be tylosed generally. Since there is no evidence presented that they were ever open, this is probably not a true tylosed condition, but rather the solid condition of the ancestral forms, a condition which is very prevalent in the older fossils. It is to be noted also that in the two forms which Miss HOLDEN considers more primitive than *P. protoscleropitys*, resin canals are said to be filled with *thick-walled* cells. In one of them, *P. foliosum*, where the resin ducts are very numerous, they are frequently in tangential groups of three or four. The fact that tangential series of resin canals can be revived in the living pines by injury, and that such traumatic resin canals are usually solid is in agreement with the fossil condition, and indicates that the resin ducts of the pines were ancestrally of this type.

In all three forms, the pits are usually uniseriate and scattered. In *P. protoscleropitys*, Miss HOLDEN speaks of the terminal pits of the tracheids as being sometimes "closely approximated and flattened by mutual contact." She has not described this pitting in the other two. No bars of Sanio have been observed except in the former, the most specialized form. The ray pitting of its tracheids, too, shows a tendency toward the formation of "Grosseiporen," while theirs is piciform, the more primitive condition. Tangential pitting of the summer wood is absent in *P. protoscleropitys*, but present in the others. She says that this confirms "the conclusions of JEFFREY and CHRYSLER that tangential pitting is a primitive feature now lost in the more highly specialized hard pines." She has evidently overlooked STRASBURGER'S previous statement of this conclusion (Hist. Beiträge **3**:9. 1891), as did JEFFREY and CHRYSLER themselves.

Miss HOLDEN'S *P. protoscleropitys* has the sculptured ray tracheids of a hard pine, while the other two forms have no ray tracheids. She has looked in the former for verification of the mode of origin of ray tracheids proposed by THOMPSON,[11] from vertically elongated tracheary elements, but on finding none disparages the correctness of THOMPSON'S work, stating that it is "unlikely that this hypothesis is correct." She has evidently not understood the problem, for the form in which she looked for evidence is, by her own statement, a specialized one in this very feature. Again, she was dealing only with the *stem*, while THOMPSON worked chiefly with the more conservative organ, the *root*. Moreover, the recent investigations of CHRYSLER,[12] who has thoroughly worked over the ground from the standpoint of the phloem, have confirmed THOMPSON'S conclusion. He found the evidence in the root so much clearer than in the stem that he says he soon discontinued the study of the latter.—R. B. THOMSON.

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[11] THOMPSON, W. P., The origin of ray tracheids in the Coniferae. BOT. GAZ. **50**:101–116. 1910.

[12] CHRYSLER, M. A., On the origin of erect cells in the phloem of the Abietineae. BOT. GAZ. **56**:36–50. 1913.

A new interpretation of mitosis.—In 1911 DEHORNE published two papers[13] setting forth a new interpretation for the phenomena of somatic and heterotypic mitosis in animals and plants. According to this author, the units usually called chromosomes are in all stages of all divisions associated in pairs, each pair having the value of a longitudinally split single chromosome. At metaphase they are not divided along this split, but are simply separated into two groups which pass toward opposite poles. During anaphase the members of each pair separate somewhat from each other and become secondarily split. After persisting through the resting stages as interlaced spiral threads, these two double structures are finally separated at the next metaphase. Thus the line of separation at any metaphase is determined during the second preceding anaphase. The diakinetic pairs are in like manner regarded as longitudinally split somatic chromosomes. At the first maturation division one-half of these pairs goes to each pole, bringing about a reduction. During anaphase each member is longitudinally split as in the somatic mitoses. At the second division instead of separating into their longitudinal halves, they are distributed in two groups of double rods. According to this interpretation the haploid number of chromosomes in *Lilium* should be regarded as 6 and the diploid number as 12, rather than 12 and 24.

GRÉGOIRE,[14] in a very detailed description of the metaphase and anaphase in *Galtonia*, *Trillium*, and *Allium*, demonstrates clearly that in every case a dicentric separation of the halves of each chromosome occurs, and that there is no such pairing as DEHORNE has described. In a second short note[15] he shows, after a careful study of *Lilium*, that the phenomena of maturation follow the heterohomeotypic scheme previously described by him, and contradict in all points the conclusions of DEHORNE. What is true of *Lilium* is held by GRÉGOIRE to be generally true of all higher plants and many animals. These results, together with those of MUCKERMANN[16] on *Salamandra* and other forms, are conclusive in showing that the interpretation of DEHORNE is wholly false.—L. W. SHARP.

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[13] DEHORNE, A., Recherches sur la division de la cellule. I. Le duplicisme constant du chromosome somatique chez *Salamandra maculosa* Lour. et chez *Allium Cepa* L. Archiv f. Zellforschung **6**:613–639. *pls. 35, 36. figs. 2.* 1911; Recherches sur la division de la cellule. II. Homéotypie et hétérotypie chez les Annélides polychètes et les Trémotodes. Arch. Zool. Exp. et Gén. **9**: 1911.

[14] GRÉGOIRE, V., Les phénomènes de la métaphase et de l'anaphase dans la caryocinèse somatique. À propos d'une interprétation nouvelle. Annales Soc. Sci. Bruxelles **34**:pp. 36. *pl. 1.* 1912.

[15] ———, La vérité du schéma hétérohoméotypique. Compt. Rend. **155**:1098–1100. 1912.

[16] MUCKERMANN, H., Zur Anordnung, Trennung, und Polwanderung der Chromosomen in der Metaphase und Anaphase der somatischen Karyokinese bei Urodelen. La Cellule **28**:233–252. *pls. 2.* 1912.

Soil moisture measurement.—The water content of the soil has long been recognized as the most important edaphic factor in limiting the occurrence and permanence of plant associations, but it has always been difficult to measure such a factor in terms that could be related to plant production. CRUMP,[17] in his studies of the vegetation of peat soils, has devised a method of expressing the relative humidity of these soils in such a manner that a definite index of water as an ecological factor is obtained. This index he has termed the "coefficient of humidity," and it seems, for the habitats studied, to be a constant whose value may be determined for any given plant association. In obtaining this constant, the amount of water present in any soil is expressed in terms of percentage of the dry weight at 15° C., and the humus-content being determined in the usual way by combustion, the ratio of the water-content is obtained in terms of the humus-content as follows:

$$\frac{\text{water-content}}{\text{humus-content}} = \text{coefficient of humidity}$$

This coefficient is shown to vary directly with the amount of water available for the use of the vegetation of a habitat, and the investigator believes it to be a true integration of the relative humidity of the soil of different areas. He admits, however, that his methods will not apply to sandy soils with small humus-content, and probably not to many clays, although he has devised a correction which permits it to be used for sub-peats containing large amounts of sand.

Applying this unit of measurement to certain moor plant associations, he finds[18] that the mean coefficients of humidity for the *Eriophorum* moor, the *Calluna* moor, and the *Molinia* moor of the Southern Pennines to be respectively 6, 3.3, and 2; and thus he is able to institute a direct comparison between the water conditions of these associations and others in the same formation.

It would seem that as the result of these investigations the ecologist has been given a most important method of expressing soil moisture, far in advance of anything before available, and it is to be hoped that it will be found to be applicable to a great variety of soils.—GEO. D. FULLER.

Chromosomes in Allium.—In the nuclei of *Allium Cepa* BONNEVIE[19] has described a large chromatin knot from which the chromatin threads radiate. In the presynaptic stages in the pollen mother cells these threads become paired. From a comparison with the origin and behavior of similar radial threads in

[17] CRUMP, W. B., The coefficient of humidity: a new method of expressing the soil moisture. New Phytol. **12**:125–147. 1913.

[18] CRUMP, W. B., Notes on water content and the wilting point. Jour. Ecol. **1**:96–100. 1913.

[19] BONNEVIE, K., Chromosomenstudien. III. Chromatinreifung in *Allium Cepa* (♂). Arch. f. Zellforschung **6**:190–253. *pls. 10–13.* 1911.

the somatic divisions, the conclusion is drawn that this process represents a side-by-side conjugation of somatic chromosomes, which are separated at the first maturation division.

MOTTIER and NOTHNAGEL,[20] after a study of the pollen mother cells of *Allium cernuum*, come to very different conclusions regarding the conjugation process. These stand in agreement with the earlier accounts of MOTTIER, and may be summarized as follows. Synapsis is a real contraction of the linin net with its chromatin granules. The thick spirem which emerges therefrom shows only an occasional temporary split in some parts. Nothing is found to indicate a union of two spirems in the prophases. During a second contraction the spirem is thrown into loops, and cross-segmentation occurs. The bivalent chromosomes so formed are regarded as consisting of two somatic chromosomes previously arranged end to end in the spirem. The members of each bivalent separate at the first division and during anaphase become longitudinally split in preparation for the second. At telophase there is formed an interrupted spirem, but there is present no chromatin knot such as BONNEVIE has described and which MOTTIER and NOTHNAGEL believe to be due to improper fixation.

The above works recall the earlier researches of BERGHS[21] and of GRÉGOIRE[22] on *Allium fistulosum*, in which they found the bivalent chromosomes arising through a union of two spirems in the presynaptic or synaptic stages, as BONNEVIE later found in *Allium Cepa*. The figures given by these writers to illustrate these critical stages form a much more complete series than those accompanying the contribution of MOTTIER and NOTHNAGEL. It is hardly probable that the disagreement between these accounts is due entirely to the fact that different species of *Allium* were used.—L. W. SHARP.

Cytology of mutants.—Some of the cytological aspects of the *Oenothera* question are summarized by GATES[23] in a discussion of tetraploid mutants. *O. gigas*, a tetraploid form, in all probability arises through the apogamous development of an unreduced megaspore mother cell with 28 chromosomes ($4x$), such a cell having been seen by GEERTS. Triploid mutants seem to be due to the union of a diploid with a haploid germ cell (STOMPS, Miss LUTZ). In some plants the mutational changes are not confined to the meiotic divisions, but at

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[20] MOTTIER, D. M., and NOTHNAGEL, M., The development and behavior of the chromosomes in the first or heterotypic mitosis of the pollen mother cells of *Allium cernuum* Roth. Bull. Torr. Bot. Club **40**:555–565. *pls. 23, 24.* 1913.

[21] BERGHS, J., La formation des chromosomes hétérotypiques dans la sporogénèse végétale. II. Depuis la sporogonie jusqu'au spirèm définitif dans la microsporogénèse de l'*Allium fistulosum*. La Cellule **21**:383–394. *pl. 1.* 1904.

[22] GRÉGOIRE, V., La formation des gemini hétérotypiques dans les végétaux. La Cellule **24**:369–420. *pls. 2.* 1907.

[23] GATES, R. R., Tetraploid mutants and chromosome mechanisms. Biol. Centralbl. **33**:93–99, 113–150. *figs. 7.* 1913.

many different stages irregularities in chromosome distribution may occur in a variety of ways. GATES believes several characters of *O. gigas* cited by DE VRIES as occurring independently of chromosome doubling are the result of the tetraploid condition with its larger cells and nuclei. Many such differences are attributed to causes fundamentally quantitative. The interpretation of NILSSON, that *O. gigas* originates by the accumulation of factors for size, is held by GATES to be contradicted by the cytological facts and by the sudden origin of giant types with their subsequent wide variation. Although some *Oenothera* characters are Mendelian in their behavior after they appear, Mendelian combinations in NILSSON's sense are inadequate to account for their first appearance.—L. W. SHARP.

A heterosporous fern.—LIGNIER[24] has published a new genus (*Mittagia*) from the Lower Westphalian strata, which is the first heterosporous fossil fern to be described. That such a group did occur is, of course, postulated by the existence of the seed ferns, but this is the first demonstration of its presence. LIGNIER, too, has sounded a note of warning by his discovery. He was at first inclined to consider that his sections were of a pteridosperm, *Lagenostoma Lomaxi*, so similar in structure are the outer tissues of the sporangia in the two forms. When he found four megaspores to a sporangium, a stomium present, and the sporangia arranged in a sorus, he knew that he had something different. His sporangium, however, he considers did not dehisce, and so, like *Lepidocarpon*, is a stage toward the seed habit. His conclusion that the sporangia belonged to a fern is based chiefly on their structural resemblance to *Lagenostoma*, and on their arrangement in a sorus. He has further distinguished them from the lycopod and equisetum lines, from *Lepidocarpon*, *Miadesmia*, *Selaginella*, heterosporous calamites, etc. LIGNIER's intensive study of the small amount of material at his disposal and his logical deductions are exceedingly interesting and valuable.—R. B. THOMSON.

Evaporation in Skokie Marsh.—Using the Livingston atmometer, SHERFF[25] measured the evaporating power of the air in a marsh habitat near the city of Chicago during the summer of 1911. The average daily rate of evaporation for the lowest stratum of vegetation was 3 cc. for the *Typha* association, 4.27 cc. for the reed swamp, 4.5 cc. for the swamp meadow, and 7.9 cc. for the swamp forest of *Quercus bicolor* and *Fraxinus americana*. This forest is normally antecedent to a truly mesophytic forest such as that found by the reviewer to have an average daily rate of 8.1 cc.[26] During September and October of the

[24] LIGNIER, O., Un noveau sporange séminiforme. Mém. Soc. Linn. Normandie **24**:49–65. 1913.

[25] SHERFF, E. E., Evaporation conditions at Skokie Marsh. Plant World **16**:154–160. 1912.

[26] BOT. GAZ. **52**:193–208. 1911.

same season SHERFF also obtained data upon the evaporation rates in different strata of the marsh vegetation, showing the evaporating power of the air to be 300 per cent greater in the top stratum of the *Phragmites* association than in the lowest, while the difference became three times as great in the *Typha* association. These results confirm those of YAPP[27] for a sedge vegetation and those of the reviewer[28] for the beech-maple forests, warranting the conclusion that plants may grow in proximity with each other and yet, vegetating in different horizontal strata, be subject to widely different growth conditions.—GEO. D. FULLER.

History and origin of monocotyledons.—HORWOOD[29] has done useful service in bringing together, in convenient form, the evidence of fossil monocotyledons. The record of each family is recited, and the summary shows that the first authentic specimens are from the Cretaceous, and that in the Tertiary or Post-Tertiary 24 families out of about 30 are represented. In dealing with the origin of monocotyledons, HORWOOD gives a synopsis of most of the views that have been advanced and reaches the following general conclusion: that the monocotyledons and dicotyledons are divergent series from a common ancestor; that among the dicotyledons there has been "progression and differentiation," while among the monocotyledons there has been "retrogression and even some reduction from a common ancestor of the primitive angiospermic type." This primitive type, by the way, "resembled an alismaceous or liliaceous type, on the one hand, and a ranalian type on the other," and in the background of this primitive stock the author sees the Cycadafilicales and Bennettitales. The mass of facts brought together will be very useful, even if the conclusions are not convincing.—J. M. C.

Fourth International Botanical Congress.—The first circular of the International Botanical Congress of 1915 has been issued. The sessions will be held in London from May 22 to May 29. Membership is secured by subscribing to the regulations of the congress and by the payment of a subscription of 15 shillings. Ladies accompanying members may attend the meetings and excursions of the congress on payment of 10 shillings each. The presidents of the organizing committee are Professor F. O. BOWER, Sir DAVID PRAIN, and Professor A. C. SEWARD. The general secretary is Dr. A. B. RENDLE, British Museum, Cromwell Road, London, S.W., to whom applications may be made.

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[27] YAPP, R. H., On stratification in the vegetation of a marsh, and its relations to evaporation and temperature. Ann. Botany **23**:275–320. 1909.

[28] BOT. GAZ. **54**:424–426. 1912.

[29] HORWOOD, A. R., The past history of monocotyledons, with remarks on their origin. Scottish Bot. Review **1**:164–180, 216–234. *pls. 1–4*. 1912.